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## THE METABOLISM OF THE SALIVARY GLANDS.

### III. The blood sugar metabolism of the Submaxillary Gland.

By G. V. ANREP AND R. K. CANNAN (*Beit Memorial Research Fellow*)

(*From the Institute of Physiology, University College, London*)

IN a recent communication (1) we have reported the results of a study of the consumption of blood sugar by the submaxillary gland both in the resting condition and when secreting under the influence of pilocarpine. The experiments demonstrated that the resting gland consumes blood sugar at a fairly constant rate and that this rate is greatly increased under the influence of pilocarpine—the increase being in broad proportion to the activity of the gland as measured by the flow of saliva. The present paper contains a report of experiments upon the effect of stimulation of the chorda tympani together with a further study of any possible relation between the rate of flow of blood through the gland and its consumption of blood sugar. This latter question arose from the fact that activity of the gland being usually accompanied by a considerable acceleration of the blood flow it was necessary to determine if this acceleration were responsible for the increased disappearance of sugar. That our results were governed by any such relation was rendered very improbable by an inspection of the collected protocols of the experiments. Thus if we take for each observation the factor of increase of consumption consequent upon stimulation of the chorda and divide it by the factor of increase in blood flow we obtain ratios ranging impartially between 0.3 and 3.2. The variation in any one animal was never so great as this but was often considerable. It is difficult to see how any direct relation can exist between these two factors when they show such wide degrees of individual variation. Experiments directly designed to answer this question were also undertaken, a study being made of the changes in the consumption of the resting gland under conditions of artificially varied blood flow.

*Diminution of the blood flow.* It was thought that the simplest way to reduce the blood flow through the gland would be to clip the corresponding carotid artery but upon performing the experiment it was found that no definite reduction of flow generally resulted from the

from making any such calculation for his experiments upon dogs under pilocarpine or chorda stimulation.

*The atropinised gland.* The effect of chorda stimulation of the atropinised gland was found to be in good accord with the results of Barcroft(5) on the oxygen consumption and those of Gesell(6) on the electrical variation of the gland. Atropine in doses sufficient to abolish visible secretion gave values for sugar consumption which were above those determined for the resting gland but less than would be expected for the secreting gland. With larger doses, however, though vaso-dilatation was still produced on stimulation of the chorda, there was found to be no increase in the consumption of sugar. In some cases a given dose of atropine fully paralysed the gland for a time giving normal resting values for sugar consumption but later, though no visible secretion appeared on stimulation, yet the sugar consumption was seen to be increasing.

Exp. 6.

Time in minutes ...	0	16	35	44	54	59	72
Blood flow c.c. per min.	0.49	0.56	5.4	0.91	5 mgms. of atropine injected here	0.82	7.14
Consumption (mgms. per gm. of gland per min.)	1.9	2.1	10.2	2.2		2.4	1.9
Stimulation of chorda ...	...	...	+	...		...	+
Time in minutes ...	79	95	97	107	180	185	
Blood flow c.c. per min.	0.61	3.0	1.6	0.52	2.38	0.48	
Consumption (mgms. per gm. of gland per min.)	2.1	1.8	4.9	1.7	4.6	2.3	
Stimulation of chorda ...	...	+	+	...	+	...	

It is evident that the dose of atropine abolished the increase in sugar consumption for a time only. Eighteen minutes after injection a stimulus sufficient to increase the blood flow ninefold was without effect on the consumption of sugar. After a further period of twenty-three minutes, stimulation for two minutes gave no increase but an observation made immediately after the first without interrupting the stimulation gave a significant increase—that is to say, after a latent period of two minutes. A third observation made two hours after injection of the atropine gave an immediate rise in consumption. The following experiments on the effect of a large and of a small dose are contrasted.

Exp. 7. Large dog. 2.5 mgms. injected when the preparation was ready.

Time ...	0	45	54	63	70	80	114	130
Blood flow ...	1.20	2.85	2.85	1.47	1.20	1.52	0.53	1.78
Consumption ...	3.3	6.9	6.8	3.5	2.7	6.9	2.1	4.6
Chorda ...	...	+	+	weak	...	+	...	+

The small dose of atropine was insufficient to prevent some increase in sugar consumption but the increase was always smaller than, under similar conditions, was found for non-atropinised glands.

Exp. 8. Small dog. 7.0 mgms. were injected at 82 minutes.

Time	...	...	0	13	23	33	82	91	103	107	115	140	155
Chorda	...	...	...	...	+	...	...	...	+	...	...	+	...
Saliva c.c. per min.	...	...	...	...	0.14	...	...	...	...	...	...	...	...
Blood flow	...	0.86	0.88	1.30	0.66	...	...	1.14	1.60	1.00	1.14	4.00	0.72
Consumption	...	3.0	2.6	8.8	3.6	...	...	5.0	2.2	5.0	5.0	1.6	4.4

The large dose here abolished all effect of stimulation on consumption throughout the experiment. Indeed a peculiarity not observed any other time was the actual diminution of the consumption.

*Sugar consumption in the period immediately following activity.* The work of A. V. Hill and of others has clearly shown that the first phase of muscle contraction is of an unoxidative nature, oxidation being a restorative process in the post-contraction period. It is, of course, impossible to distinguish in the gland the two phases of activity as clearly as in a single muscle contraction. Barcroft and Kato(4) showed for the submaxillary gland and for the tetanised muscle that the maximum oxygen consumption does not correspond with the period of maximal activity but shows a considerable lag. It is postulated(7) that the oxygen used in this latter period is consumed in the oxidation of the products of the unoxidative metabolism of the actual activity and here the cleavage of carbohydrate into lactic acid plays a large part. We were led by these considerations to determine the sugar consumption of the gland in the period immediately following activity as determined by the cessation of the secretion. The results were quite definite. There was never any indication of an increased consumption in the post-active period. The following experiment is representative of the results obtained.

Exp. 9. Time	...	0	2	4	6	8	80	82	84
Blood flow	...	2.22	7.7	7.7	1.9	1.25	5.71	1.75	1.15
Saliva	...	...	0.9	0.59	...	...	0.29	...	...
Consumption	...	6.2	28.0	21.3	4.4	6.1	13.3	6.0	6.0
Chorda	...	...	+	+	...	...	+	...	...

The post-active periods, which were at 6 and at 82 minutes respectively, were taken immediately the secretion stopped, *i.e.* only a few seconds after the removal of the stimulus. The resting consumption was in this case exceptionally high but its return to normal immediately upon the cessation of secretion is quite clear. Further indirect evidence in favour of this conclusion is to be found in the fact that in any one active period of long duration, such as that induced by the injection of pilocarpine, the consumption of sugar per 1 c.c. of saliva remains fairly constant throughout and shows no tendency, as does the consumption of oxygen, to progressively increase. We may, therefore, conclude that there are two phases in the activity of the gland—the unoxidative phase

involving the breakdown of sugar and the recovery phase in which the products of this breakdown are removed.

In conclusion, it would appear that whilst there is, in the resting gland, substantial agreement between the oxygen and the blood sugar consumption the same cannot be said, from the evidence at present available, for the respective increases in these two factors during activity. In this connection it is realised that no balance sheet of carbohydrate metabolism in the secreting gland is complete that does not account for changes in blood sugar consumption due to (a) changes in the glycogen content of the gland, (b) changes in lactic acid or similar non-reducing carbohydrate metabolites in the blood, (c) contribution of sugar to the synthesis of mucin.

The results of work published by one of us (G. V. A.) in another paper in the present number of this journal would appear to discount effectively the latter possibility, so that, bearing in mind also the conclusions of Meyerhof and of A. V. Hill that in the first phase of muscle contraction three or four times as much glycogen is converted into lactic acid as is subsequently oxidised, a study is now being made of the changes in the glycogen content of the gland and of the lactic acid of the blood during activity.

#### CONCLUSIONS.

1. Changes in the blood flow through the submaxillary gland do not affect the consumption of sugar from the blood.
2. Stimulation of the chorda tympani increases the blood sugar consumption. The increase is of the same order as that of the gland secreting under pilocarpine and is broadly proportional to the activity as measured by the rate of secretion.
3. The maximal consumption of sugar corresponds with the maximal secretion and does not occur, as does the oxygen consumption, in the post-active period.
4. Atropine in doses sufficient to paralyse the secretion only reduces the effect of the chorda tympani on the sugar consumption. Large doses abolish the effect altogether.

The expenses of this research were defrayed by a grant from the Medical Research Council.

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# THE METABOLISM OF THE SALIVARY GLANDS

## IV. The metabolism of the reducing substance of the Submaxillary Gland. By G V ANREP

*(From the Institute of Physiology, University College, London)*

Mucin is the chief product of the synthetic activity of the submaxillary gland (dog). It is also the chief form in which nitrogen is excreted by that gland during activity. In a previous communication (1) some experiments were described which were performed with the view to studying the nitrogenous metabolism of the submaxillary gland under chorda stimulation. The conclusion of those experiments was that mucin is not produced during the stimulation of the chorda, and that the synthetic activity of the gland does not take place during the periods of activity but at some other time and under other conditions. This conclusion being of general importance for the comprehension of the process of reconstruction of the glandular tissue was verified in the experiments described below.

Mucin contains in its molecule a reducing substance (known to be glucosamine), and can be traced not only by the nitrogen determination but also by its reducing substance which it yields upon acid hydrolysis.

*Method of estimating the reducing substance.* No suitable method was found in the literature on the subject for quantitative determination of a reducing substance in tissues and in presence of large amounts of protein, except those methods which are used for the estimation of glycogen and which are based on alkali hydrolysis—a process unsuitable for glucosamine. The first attempts were made with the estimation of the reduction in neutralised acid hydrolysates of the glands, as generally recommended for saliva and for pure mucin. This method, however, gave extremely inconsistent results as the reduction was found to vary within wide limits, dependent on the strength of acid used and on the length and intensity of the hydrolysis. The glands were hydrolysed in hydrochloric acid varying in strength from 3 to 10 p.c., and it was found that after the same length of time the hydrolysates from the action of the weaker acids gave a much larger reduction than those from the strong acids. In one case, for instance, the submaxillary tissue was hydrolysed for 7 hours in 3, 5 and 10 p.c. acid. At the end of the hydrolysis the three lots gave a reduction of 46.6, 34.0 and 21.4 mgms.

The other important factor is the duration of the hydrolysis. When the glands are hydrolysed in 3 p.c. HCl the reduction is found to increase for the first 4 hours; for the next 3 hours it gives maximal figures and then begins to decline. As a general rule, the hydrolysates give in the early stages only a good precipitation of copper, but as hydrolysis proceeds, the Bertrand's solution assumes a greenish-yellow colour with a gradually diminishing amount of the copper precipitate. The effect resembles the familiar colour obtained with some pathological urines. With stronger acids the maximum is reached earlier but the drop is sooner and more sudden. In still stronger acids the reduction never attains the maximal level reached with a weak acid, so that the diminution in the reduction seems to begin before the whole of the mucin is hydrolysed. Acids of less than 3 p.c. strength take too long a time to break down the glandular tissue and were therefore not used. The gradual diminution in the reduction is not due to a destruction of the glucosamine. The hydrolysis of the pure glucosamine hydrochloride in acids up to 20 p.c. strength does not show any decline in reduction. But if glucosamine is hydrolysed in the presence of some tissue (pancreas and muscles were used) it behaves in the same way as the submaxillary glands and shows a decline in reduction which increases with the length of hydrolysis and the concentration of the acid used. It was supposed that the cause lay in the interfering action of products of the acid hydrolysis of the proteins, and an attempt was made to remove these before the estimation of the reduction. Alcohol, dialysed iron, trichloroacetic acid, and charcoal (nurite) were used as precipitating agencies. Charcoal was found to be most promising. Glucosamine in an acid solution is not adsorbed by nurite, but in a neutral solution it is adsorbed to a small extent.

It was found that nurite adsorbs from the hydrolysates of the glands the substances which interfered with the reduction. The water-clear filtrates gave perfect reduction. Moreover, hydrolysates which lost the reduction after a prolonged hydrolysis, showed a nearly normal reduction after treatment with charcoal. Glucosamine added to tissue hydrolysates could be quantitatively recovered.

*The comparative estimation of the reducing substance in the right and left submaxillary gland.* The preliminary experiments having given satisfactory results, the following method was adopted for the determination of the reducing substance in the glands. The submaxillary glands were dissected, as described in a previous communication, weighed, cut into small pieces and hydrolysed separately in 40 c.c. of 3 p.c. HCl for  $4\frac{1}{2}$  hours. The hydrolysates were then cooled, transferred into a 100 c.c.

volumetric flask, and diluted with distilled water up to the mark. 20 c.c. were then taken out and used for the determination of the nitrogen; the rest was mixed with about 10 gms. of nurite, corked and left to stand for about 30 minutes. At the end of this time the hydrolysates were filtered through a dried filter into a dried flask and the reduction estimated in the water-clear filtrates. There was usually enough filtrate to make duplicate determinations.

Ten pairs of resting glands were analysed in this way. The first five pairs were taken from dogs which had been used for other experiments, and the others from dogs which had been killed by bleeding. The results of the analysis are given in Table I. The weights of the glands are not given as, for the purpose of these experiments, they are not important, and the same applies to the absolute figures for nitrogen—the  $D/N$  ratio being given instead. All dogs used for these experiments were killed during a period of rest of the digestive organs.

TABLE I.

Exp.	Reducing substance in mgms. of glucose		P.c. of reducing sub- stance in the wet weight of the glands		$D/N$ ratio	
	Left	Right	Left	Right	Left	Right
1	57.0	50.5	1.72	1.68	.68	.60
2	60.3	60.8	1.77	1.72	.72	.76
3	44.4	43.3	1.71	1.73	.78	.70
4	81.0	83.0	1.84	1.90	.76	.80
5	77.5	78.5	2.13	2.10	.76	.74
6	142.8	140.4	2.26	2.20	.85	.83
7	83.5	88.0	2.41	2.35	.84	.82
8	53.2	51.0	2.30	2.26	.80	.83
9	95.6	92.3	2.34	2.33	.80	.81
10	162.8	167.2	2.35	2.29	.83	.81
Total	864.1	870.0	Mean 2.08	2.06	Mean .78	.79

As mentioned above, the first five pairs of glands were taken from dogs used for other experiments, and they can, therefore, not be considered as absolutely resting glands. The second five pairs may be considered to be in a more truly resting condition. One conclusion is certain, and that is that the left and the right submaxillary glands have the same amount of reducing substance and that therefore one gland can be taken as a control representing the amount of reducing substance that the other gland had before activity. The percentage of the reducing substance and the  $D/N$  ratio are fairly constant; 2.3 p.c. would be more accurate for a resting gland than the mean of the ten experiments.

*The nitrogen and the reducing substance in the saliva.* Experiments were performed with the object of determining the form in which



nitrogen is secreted in the saliva. In the experiments described previously acetic acid was used to precipitate the mucin of the submaxillary saliva. All the nitrogen which was not precipitated was called non-mucin nitrogen. It was found, however, that all this nitrogen consists entirely of non-protein nitrogen and that it is immaterial whether the mucin is precipitated by acetic acid or alcohol. The acetic acid filtrates do not give protein reactions or precipitations with any of the protein precipitants. The estimations of the nitrogen in the alcoholic and in the acetic acid filtrates give identical figures. This would at first suggest that the submaxillary saliva of the dog is free from any protein but mucin. Such a conclusion is however not justified. Acetic acid and alcohol produce totally different precipitates in the saliva. The acetic acid produces a large viscid lump which only gradually shrinks and settles in a diluted saliva into a mass at the bottom of the flask. The supernatant fluid is clear and does not give precipitation on boiling or treating with alcohol or trichloroacetic acid. The alcoholic precipitate consists of two parts, one which floats on top of the liquid and consists of mucin, and the other finely flocculent which on standing settles at the bottom. This precipitate gives protein reactions and behaves like a globulin towards magnesium sulphate. This globulin was not studied more closely. No other proteins were found in the submaxillary saliva of the dog, and as mucin behaves towards magnesium sulphate like a globulin, all the proteins could be precipitated by full saturation of magnesium sulphate. It was pointed out by Hammarsten(2) that mucin when precipitated by acetic acid carries down other proteins. The small amount of globulin in the saliva which is presumably derived from the demilunes is completely carried down by the precipitate of mucin. An attempt is now being made to precipitate this globulin separately from mucin by the use of trichloroacetic acid as the latter does not precipitate mucin.

On account of this admixture of an unknown quantity comparison of the nitrogen and of the reduction values of the saliva is scarcely permissible. As a matter of fact in any given experiment the  $D/N$  ratio of the saliva always diminishes in the later stages of secretion. The percentage of the reducing substance also diminishes as the saliva becomes poorer in mucin as can be seen from the following experiment.

Exp. 11. 90.5 c.c. of submaxillary saliva were obtained by the stimulation of the chorda tympani. The saliva was collected in two lots, first of 33.5 c.c. and then of 57 c.c.

	P.c. of reducing substance	$D/N$ ratio
1st portion	.175	2.68
2nd „	.049	1.64

If we take glands which are not in a resting condition, as in Expts. 13 and 21 of Table II, with a  $D/N$  ratio of the control glands of .52 and .49 respectively, we find, as would be expected, a low percentage and a low  $D/N$  ratio of the saliva secreted.

TABLE II.

Exp.	12	13	14	15	16	17	18	19	20	21	22
of saliva per m. of gland	10.7	10.7	11.7	13.4	14.9	18.0	21.8	22.3	22.9	27.2	42.0
ms. % reduc. ion in saliva	191	128	186	154	102	91.5	86.0	79.5	78.0	41.5	32.9
$\sqrt{}$ ratio of the saliva ...	2.60	1.07	2.85	—	2.48	1.94	1.63	1.76	1.76	1.22	1.69
$\sqrt{}$ ratio of the proteins in saliva ...	3.63	3.35	3.63	—	3.7	3.23	3.00	3.07	3.03	2.65	3.9
$\sqrt{}$ ratio of the resting gland	0.82	0.52	0.83	—	0.76	0.82	0.82	0.78	0.82	0.49	0.50

If these two experiments (13 and 21) be excluded, one finds a gradual decline in the percentage of the reducing substance in the saliva as well as in the ratio reducing substance : total nitrogen. The decline in the latter factor shows that with secretion the admixture of non-reducing nitrogenous substances increases.

The ratio reducing substance : protein nitrogen is somewhat more constant. The small variations in this ratio indicate the presence of a variable amount of protein other than mucin.

*Is the reducing substance formed by the gland during secretion?* The experiments were carried out in the same way as described in the first communication. After the stimulation of the chorda tympani the glands were dissected, freed from all connective tissue, and weighed. They were then cut into small pieces and hydrolysed in 40 c.c. 3 p.c. HCl for 4½ hours. The saliva was generally diluted to a known volume and a part also hydrolysed in 3 p.c. HCl for the same length of time as the glands. The hydrolysates of the two glands were always different in appearance, that of the resting gland being coloured dark brown with humin, that of the other, opalescent and only slightly brown. The three hydrolysates were then treated as described before. The results of eleven experiments are given in Table III which hardly needs explanation. Columns 6 and 7 should be compared. The former gives the absolute amount of reduction in mgms. of glucose found on the active side, i.e. active gland plus saliva; the latter column gives the reduction by the resting side. The differences between the corresponding figures of the two columns are well within the limits of the experimental error and do not suggest a production of

reducing substance by the gland during chorda stimulation. The experiments in Table II are the same as in Table III. The  $D/N$  ratio of the resting gland which is given in column 8 serves only to show the state of the glands at the beginning. All the glands had a  $D/N$  ratio between .76 and .83, except in Exps. 13 and 21, which showed a marked exhaustion from the beginning.

TABLE III.

1 Exp.	2 Weight of gland in gms.		3 Volume of saliva in c.c.	4 Reduction in mgms. of glucose				8 $D/N$ ratio of the resting gland
	Active	Resting		Active gland	Saliva	Sum of cols. 4 & 5	Resting gland	
12	3.72	3.93	42	12.0	80.0	92.0	93.5	0.82
13	2.39	2.43	26	7.5	33.4	40.9	39.8	0.52
14	3.27	3.69	43	6.5	80.1	86.6	85.2	0.83
15	4.95	5.36	72	11.0	110.8	121.8	125.0	—
16	2.93	3.42	51	10.5	52.0	62.5	62.0	0.76
17	2.38	2.81	53	14.0	48.5	62.5	63.6	0.82
18	2.47	3.20	70	12.0	60.2	72.2	75.0	0.82
19	2.42	2.78	62	10.0	49.3	59.3	58.3	0.78
20	2.62	3.05	70	11.5	54.5	66.0	64.5	0.82
21	2.28	2.57	70	7.0	29.2	36.2	35.4	0.49
22	3.52	3.96	166	trace	86.6	86.6	87.3	0.80

A few experiments were made with extraction of the mucin with distilled water and re-precipitation with diluted  $HCl$ , as recommended by Hammarsten(2). For quantitative estimations it was found to be unsuitable as the extraction takes too long a time and mucin undergoes decomposition. Moreover, during the re-precipitation it is difficult to avoid some loss of mucin. One point of interest was noticed—the extracts of the resting glands were extremely viscid and yielded a large precipitate on addition of an excess of acetic acid. The extracts of the exhausted glands were quite watery and gave none or very little precipitate.

Anrep and Cannon(3) have demonstrated that during the secretion of saliva the submaxillary gland consumes an increased amount of sugar from the blood. This sugar is not passed into the saliva because the saliva does not contain sugar in determinable amounts. Further, from the experiments described in this communication it is evident that it is not used to form the reducing part of the mucin excreted.

#### SUMMARY.

1. The use of nurite is advised for the removal of the products of acid hydrolysis interfering in the estimation of the reducing substance of the submaxillary glands.

2. The resting submaxillary gland yields on acid hydrolysis about 2.3 p.c. of reducing substance calculated as glucose.

3. The ratio reducing substance : total nitrogen in a resting submaxillary gland is about .8.

4. The glands on both sides yield after acid hydrolysis the same absolute amount of reducing substance.

5. The saliva secreted under chorda stimulation becomes gradually poorer in reducing substance. The  $D/N$  ratio of the saliva also drops with the extent of secretion. The ratio reducing substance . protein nitrogen is more constant. The variations in the latter indicate the presence in saliva of variable amounts of protein which does not give rise to reducing substance.

6. The submaxillary gland loses the same amount of reducing substance as is secreted in the saliva.

7. There is no evidence of a process of reconstruction of mucin during the secretion under chorda stimulation, as judged by the reduction after acid hydrolysis.

The expenses of the research were defrayed by a grant from the Medical Research Council.

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STUDIES ON THE RESPIRATION AND CIRCULATION  
IN THE CAT. II. The oxygen in the venous blood. By  
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*(From the Physiological Laboratory, Cambridge.)*

THE minute volume of blood passing through the thorax was measured in the first paper<sup>(1)</sup> of the present series by the method of cardiac puncture in which the "oxygen difference" between the arterial blood and the mixed venous blood is divided into the total quantity of oxygen used by the animal per minute. For this purpose it is not necessary to puncture the left ventricle in order to get arterial blood—a sample taken from any artery will answer the purpose. The question arises Can a similar simplification be discovered for the venous blood? Is there any vein in which the blood is constantly of the same percentage saturation as the blood in the right ventricle? This question led us to investigate the oxygen content of the blood in a number of typical and more or less accessible veins.

The experiments were performed on cats under urethane, the blood was abstracted from the vein in each case with a hypodermic needle attached to a 1 c.c. glass syringe in which was placed about 0.1 c.c. of potassium oxalate—the fluid at once filling up the dead space of the syringe and rendering the blood non-coagulable. The analyses were made in the Barcroft differential apparatus. It is, of course, necessary that the cat should be in good condition and not suffering from failure in any degree of the circulation. In 17 of the 31 experiments which constitute the present series the arterial blood was analysed. In one case it was under 90 p.c. saturated (87 p.c.) and was on the average 95 p.c. saturated.

The great variation in the percentage saturation with oxygen which occurs in the different veins in different animals is shown in Fig. 1. This gives all the determinations made. The percentage saturation is given according as it lay between 0 to 10 p.c., 10 to 20 p.c. and so on, and each dot represents a separate determination. The figure shows how little the blood in any one vein can be taken as representing that of the right side of the heart.

The variation in percentage saturation occurs not only in different animals but also in the different veins of any one animal. The typical examples given in Table I will serve to show the extent of this.

PERCENTAGE SATURATION	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100
INTERNAL JUGULAR VEIN						0	0	0	0	
EXT. JUGULAR VEIN					0	0	0	0	0	
BRACHIAL VEIN				0	0	0	0	0		
SAPHENOUS VEIN					0	0	0	0	0	0
FEMORAL VEIN	0	0	0	0	0	0	0	0	0	
RENAL VEIN				0	0	0	0	0	0	
PORTAL VEIN	0			0	0	0	0	0		
INF. VENA CAVA				0	0	0	0	0		
MIXED VENOUS BLOOD				0	0	0	0	0		

Fig. 1. Percentage saturation of oxygen in venous blood observed in 31 experiments in urethanised cats.

TABLE I. Percentage saturation with oxygen.

Exp.	Arterial blood	Right ventricle	Vein			
			Internal sphenous	External jugular	Internal jugular	Femoral
1	96	63	54	—	—	—
2	93	69	84	—	—	—
3	98	57	98	80	—	—
4	—	40	—	64	—	—
5	—	54	—	—	72	15
6	—	54	—	—	72	64

The figures in the table all refer to the resting animal; in many veins the range of variation would probably be greatly extended if special conditions were imposed upon special organs which they drain, such as diuresis in the kidney, contraction in muscle or the application of heat or cold to the skin, as was shown in the last case by Meakins and Davies(2), and Barcroft and Nagahashi(3). In the present cases the high saturations in the saphenous vein are caused by loss of tone at the commencement of the experiment, due probably to the fact that the anæsthesia was initiated with A.C.E. mixture.

### CONCLUSION.

In any given vein the percentage saturation with oxygen varies widely; it varies independently in different veins in apparently similar conditions of urethane anæsthesia, and it bears no uniform relation to the blood in the right ventricle.

We wish to express our warm thanks to Prof. Langley for the facilities given us in the Cambridge laboratory, to Mr Barcroft for his kind suggestions and help and to the Royal Society which defrayed a part of the expenses of the apparatus.

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CIRCULATION AFTER CESSATION OF WORK, WITH  
SOME REMARKS ON THE CALCULATION OF CIRCULATION RATE EXPERIMENTS ACCORDING TO THE  
NITROUS OXIDE METHOD. By J. LINDHARD.

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THE experiments to be mentioned below have been performed according to the nitrous oxide method of Krogh and Lindhard(1). Since this method was published several attempts have been made to find out a method more easy to manipulate and providing, if possible, also a more reliable determination of the blood flow through the lungs. Within the last ten years a very great number of determinations of the circulation rate have been performed by means of the nitrous oxide method and a statistical treatment of the results obtained under standard conditions has shown the probable limits of error of the method to be rather narrow. The uncertainty on the single determination, including technical errors as well as physiological variation, amounts to some 10 p.c. (Lindhard(2)). Other methods which must be taken into account when discussing the determination of the circulation rate in man are the various methods based on Fick's principle. It seems, that each physiologist applies this principle in his own manner, and a statistical treatment of the results cannot therefore be undertaken at present. Nevertheless the results obtained agree on the whole very well and are also consistent with the results obtained by the nitrous oxide method, as shown in the following Table I.

The table includes results from experiments in sitting and recumbent positions. According to Lindhard(3) the results may vary with the position of the body. For the present purpose only experiments made in sitting or recumbent position are of interest. Now it has been shown in the paper cited, that men and women behave in a different manner when changing from sitting to recumbent position, as in four male subjects the circulation rate was practically unaltered, while in three females it was very much increased. This fact must of course be considered when comparing the results given in the table. Doing this we



TABLE I.

Author	No. of subjects	Sex	Position	O <sub>2</sub> per litre blood after	
				N <sub>2</sub> O method	Fick's principle
Boothby (3)	1	male	lying	52	—
"	1	—	sitting	49	—
Fridericia (4)	1	—	lying	42	46
"	1	—	sitting	65	60
Christiansen, Douglas and Haldane (5)	3	—	—	—	43 39.5 39.5
"	1	female	—	—	57
Douglas and Haldane (6)	3	male	—	—	35 53 59
Lindhard (7)	6	—	—	{ 41 <sup>1</sup> 53 53.5 52 52 53.5	—
"	4	female	—	58.5 56 56 58	—
" (8)	3	—	lying	44 40 48	—
Liljestrand and Lindhard (9)	2	male	sitting	—	65.5 60.5
Liljestrand and Stenström (10)	2	—	lying	—	51 47
Lundsgaard (11)	1	—	sitting	51.5	—
"	1	female	—	63	—
Sonne (12)	1	male	lying	—	41
"	3	female	—	—	32 40 41.5

<sup>1</sup> This subject had not quite sound lungs.

see, that the two methods give fairly consistent results. It may be seen also, however, that other factors than sex and body position have been at work. In fact the greatest variations in the results are due neither to these factors nor the method but to the subject or to the experimenter. This is most evident when we regard the experiments with the male subjects as change in position does not influence these results. The figures given in the table may be arranged as follows:

O <sub>2</sub> per litre blood	31-35	36-40	41-45	46-50	51-55	56-60	61-65
Number of series	1	2	3	3	9	2	3

There is no doubt, that the normal values are found in the column 51-55 which moreover includes the most extensive series and in the two neighbouring columns, while disturbing influences have played a part in the very high and the very low values. Thus we find very high figures for the oxygen absorption when the subject has been resting a very long time and begins to be sleepy. Under such conditions different methods applied to the same subject have given the following results:

Method	Krogh and Lindhard	Fridericia	Liljestrand and Lindhard
O <sub>2</sub> per litre blood	65	60	65.5

On the other hand we find in a subject, who does not maintain standard conditions, and especially by lack of mental quiescence in the preliminary resting period, deviations in the opposite direction and

consequently a too high circulation rate. It is therefore unjustifiable to lay special stress as Douglas and Haldane<sup>(6)</sup> do, on a few deviating experiments. There are indeed very strong reasons to believe, that the subjects concerned have not maintained standard conditions. This can be shown with certainty in other experiments published by these authors as pointed out by Krogh and Lindhard<sup>(13)</sup> and, regarding the experiments of Campbell, Douglas, Haldane and Hobson, by Lindhard<sup>(11)</sup>. The last named erroneous experiments might moreover have convinced Douglas and Haldane, that H-ion concentration is not the only factor governing the lung ventilation.

We may summarize as follows: All observers have, notwithstanding the method employed, obtained results of the same order of magnitude, it is therefore not essential which method is employed. The reliability of the results depends on the care in the performance of the experiments and on the "stability" of the subject experimented on.

The choice of method being mainly a matter of convenience it may be worth while to compare the technic of the two kinds of methods here concerned.

Measurement of the circulation rate according to Fick's principle as worked out recently by Douglas and Haldane<sup>(6)</sup> and others involves the determination of the venous and arterial  $\text{CO}_2$  tensions, the  $\text{CO}_2$  dissociation curve of the blood and the gaseous exchange of the subject. The last named function must be determined also and in the same manner when using the nitrous oxide method. The  $\text{CO}_2$  dissociation curve must necessarily, according to the curves hitherto published, especially those in the recent extensive work of Barcroft, Hill and co-workers<sup>(15)</sup>, be determined in each subject and the determination is rather laborious. Thus the curve for the subject J. L. in the paper of Liljestrand and Lindhard<sup>(9)</sup> though based on six determinations is very probably not correct. Also the curve given in the paper of Christiansen, Douglas and Haldane<sup>(5)</sup> deviates strongly from the average curve of Barcroft and Hill in shape and position. Moreover it may be regarded as doubtful, whether the dissociation curve under varied conditions will prove an individual constant. The arterial or the alveolar  $\text{CO}_2$  tension may in experienced subjects at rest be determined by the H-P-method; in untrained subjects and in all cases during muscular work this method gives, as has been shown repeatedly by Krogh and Lindhard<sup>(13, 16)</sup> experimentally, results which are fallacious and must be so for reasons which Douglas and Haldane<sup>(6)</sup> have accepted as quite logical. As a rule, therefore, the alveolar  $\text{CO}_2$

tension must be calculated, and the calculation involves a determination of the dead space. It must be born in mind, however, that the physiological difficulties are always more dangerous than are the technical ones. The alveolar  $\text{CO}_2$  tension is a highly variable function governed partly by nervous influences which may change very rapidly. Such variations may be quite "accidental" as in emotions or quite regular as on the transition from rest to work (Krogh and Lindhard(17)). Therefore, the most careful determination of the alveolar  $\text{CO}_2$  tension, when used in the calculation of the circulation rate, may lead to very erroneous results, namely, if the alveolar tension has altered in the time interval between the drawing of the alveolar sample and the determination of the venous gas tensions. This point of view has already been touched upon by Liljestrand and Stenström(10). The determination of the venous gas tensions is as a rule carried out in a quite similar manner as is the circulation rate experiment according to Krogh and Lindhard, and the determination must, therefore, of course be open to the same objections as the latter. The objections generally met with aim at two points, viz. the mixing of the gases in the lungs and the influence of the technic of the experiment upon circulation. However, the mixing of the gases in the lungs offers no serious difficulties to the experienced experimenter. Regarding the other question it cannot be denied, that the experimental technic influences the blood flow; as a rule the circulation rate is accelerated but not in a quite uniform manner. Now the breath as a rule is only held for a few seconds and the absolute increase in the blood volume due to the experiment is, therefore, very limited though not insignificant. We will consider a concrete example: Minute volume directly calculated 7.66 litres. Oxygen absorption rate during the experiment 413 c.c. per min. Oxygen consumption per min. (from respiration experiment) 314 c.c. Experimental time .235 min. In this example the conditions are rather bad owing to the great "reduction" as well as to the experimental time being unnecessarily long. We have:

$$\text{Real minute volume} = 7.66 \times \frac{314}{413} = 5.82 \text{ litres.}$$

During the experiment the blood flow through the lungs has amounted to 1.80 litres, while the normal inflow in the veins in the same interval of time has been 1.37 litres. Difference .43 litre which thus represents the increase in circulation rate caused by the experimental technic. Now we must assume, that more than half of the total blood volume is stored in the veins, to a great part in the large central veins, from which the right heart is filled and which may contain a very varying volume of

blood. If in an adult man the veins contain 2.5-3 litres of blood the drawing off of .43 litre of blood is possible without other consequences than a moderate fall in venous pressure; it is not necessary to assume an increased inflow of the same order of magnitude in the veins of blood from the capillaries which perhaps might render the determination unreliable. A more dangerous possibility is, that the aspiration of blood to the central veins might alter the composition of the blood in the right heart. This possibility seems, however, not to be realized in practice. Series of circulation rate experiments by the nitrous oxide method have shown, that the "reduction" to normal exchange, caused by the acceleration of the blood flow, has no appreciable influence on the essential figure of the determination, viz. the oxygen absorption per litre blood. The determinations of the venous gas tensions with this technic may therefore be regarded as reliable. On the other hand the "reduction" cannot of course be regarded as a drawback on the nitrous oxide method. The only difference in the two methods is, that in the method of Krogh and Lindhard the acceleration of the circulation rate may be exactly measured, while in the venous tension experiments it is quite unknown. In addition to the procedures here named the determination of the circulation rate according to Fick's principle involves a varying number of gas analyses with regard to  $\text{CO}_2$  and oxygen.

The determination of the circulation rate by means of the method of Krogh and Lindhard has been described in detail on several occasions (1, 14, 18). It includes a circulation rate experiment proper and a determination of the respiratory exchange as in the method mentioned above. The technic of the circulation rate experiment, in principle quite analogous to the determination of the venous gas tensions, has already been dealt with and requires no further comment. Particular to this method is the analysis of two gas samples with regard to  $\text{CO}_2$ , oxygen and nitrous oxide; especially the combustion of  $\text{N}_2\text{O}$  may give some trouble to the unexperienced, even later a complete analysis of this kind takes the same time as two analyses of atmospheric air.

The calculation of the result has hitherto been carried out in the manner, that the blood flow per minute and the oxygen absorption per minute have been calculated separately from the experimental data, and then the figure found for the circulation rate has been "reduced" to normal exchange by means of the oxygen consumption measured in a corresponding respiration experiment. As, however, this "reduction" has been misunderstood it will be omitted in future calculations. The only data needed from the circulation rate experiment for the calcu-

lation are the results of the two gas analyses and a barometer reading, as shown in the following example:

E.H. sitting on ergometer.  $O_2$  per minute (from respiration experiment) 266 c.c.

Sample	I	I corr.	II	Diff.	$N_2O$ tension (I + II/2)	Barometer
$CO_2$	3.90	—	5.31	—	—	—
$O_2$	14.63	15.06	12.88	2.18	—	—
$N_2O$	15.80	16.26	14.19	2.07	15.00	762

When blood is exposed to a definite volume of a gas mixture containing  $N_2O$  and  $O_2$  the volume absorbed of each gas within a definite time must be proportional to the differences in percentage of the two gases before and after absorption. In the above example we have

$$\frac{O_2 \text{ difference}}{N_2O \text{ difference}} = \frac{2.18}{2.07} = 1.05.$$

If the absorbed volume of one of the gases in a given quantity of blood can be determined, the volume absorbed of the other gas in the same quantity of blood may be calculated. Now we know, that 1 c.c. of blood absorbs .405 c.c. ( $0^\circ$ , 760 mm.) of nitrous oxide at a tension difference of 100 p.c. and normal atmospheric pressure, hence the oxygen absorption per litre blood in the case here concerned is

$$1.05 \times .405 \times \frac{15}{100} \times \frac{715}{760} \times 10^3 = 60.1 \text{ c.c.}$$

and the circulation rate  $266/60.1 = 4.4$  litres per minute.

This simplification of the calculation involves further a simplification of the whole experiment, indeed we do not want to know anything about the spirometer volume, the experimental time, the alveolar residual air of the subject or the temperature, even the spirometer is superfluous as the mixing respiration may undoubtedly be taken in a Douglas bag. It cannot, however, be recommended to reduce the apparatus too much. When using a recording spirometer the observer can see simply by a glance on the curve, if the mixing respirations are deep enough, if the two expirations sampled are sufficiently deep to secure the washing out of the "dead spaces", and, if the time elapsed from the beginning of the mixing respirations until the last deep expiration is suitably short. As the gas analyses represent most of the work it is always wanted to ascertain, whether the experiment has been successful or not, before analysing.

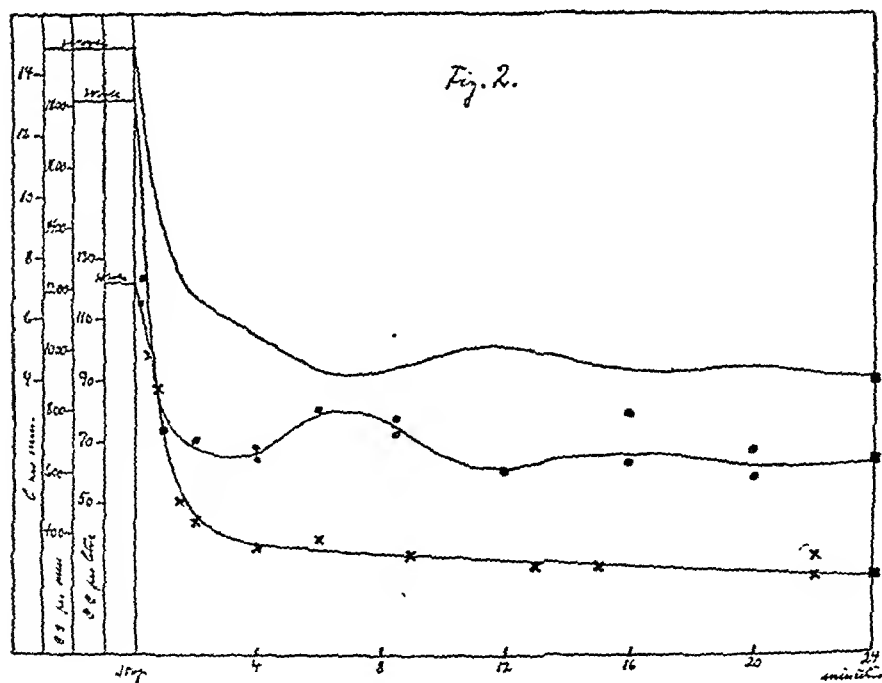
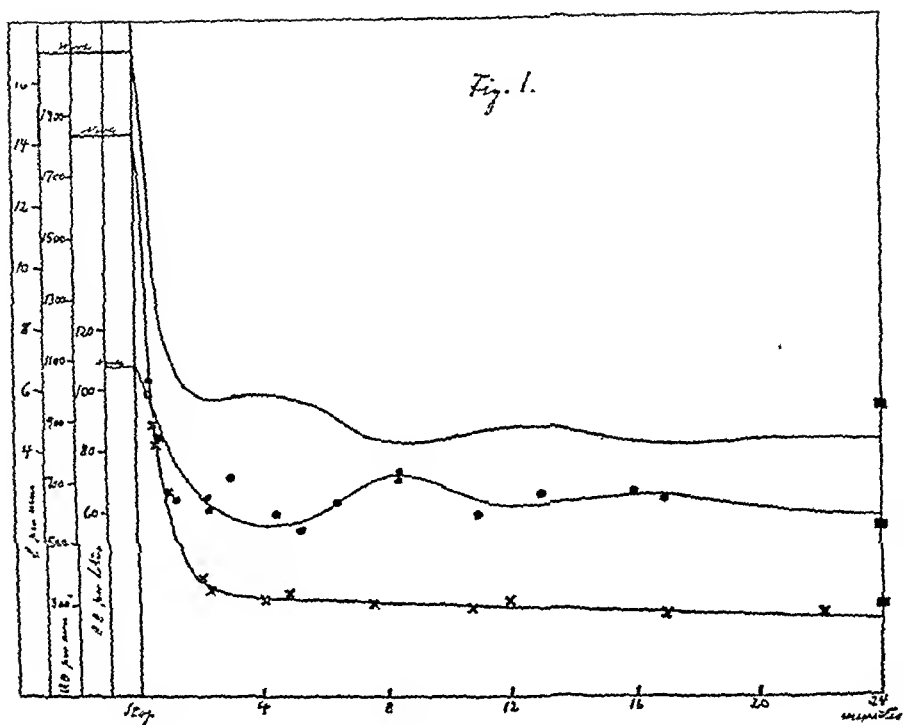
It is evident from the above, that the determination of the circulation rate according to Fick's principle is a much more complicated and laborious procedure than is the circulation rate experiment of Krogh

and Lindhard and, especially in its recent variations if the  $\text{CO}_2$  dissociation curve and the "dead space" are not determined for each subject, it is far less reliable than the latter. There is no doubt, however, that carefully performed experiments according to Fick's principle as those of Liljestrand and Stenström give quite reliable results.

When comparing the two methods it must be taken into account further, that the various procedures necessary for the Fick-determination cannot be made simultaneously but successively which involves, that corresponding values of the functions concerned can only be obtained when the physiological equilibrium of the subject remains unaltered within a definite space of time. The Fick-methods cannot, therefore, be applied if one or other of the functions concerned is varying as *e.g.* in the transition from rest to work and *vice versa*. The nitrous oxide method, on the other hand, permits us to perform an experiment so to say in a moment; it is the only method by the aid of which it is possible to follow the variations in a labile function like the circulation rate. This method was, therefore, the only one available in the investigations dealt with in the present paper.

The experiments here given may be regarded as a supplement to previous experiments of Krogh and Lindhard (19). The experiments were performed on two subjects very familiar with the work on the bicycle ergometer. The work done was in both cases 690 kpm. per minute and lasted 25-30 minutes. Shortly before work was stopped the pulse rate was counted in the usual clinical manner, the respiratory exchange determined by means of a Douglas bag and the circulation rate determined. The resting level of the functions named was ascertained with the same methods when the fasting subject sitting on the ergometer had rested for about half-an-hour. In the transitional period corresponding values of the functions investigated cannot be obtained on a single day but each function must be represented independent of the others by a curve built up of observations from day to day. When two circulation rate experiments were performed on the same day there was always allowed a suitable interval between them to make certain that all nitrous oxide from the first experiment had been expired before the second began. To avoid getting nitrous oxide into the Douglas bags the respiratory exchange was as a rule determined before the circulation rate experiment.

The *respiration experiments* are, as regards the oxygen consumption, plotted in Figs. 1 and 2 (lower curves); they have been carried out by means of Douglas bag, Lovén valve and Zuntz mouthpiece and the



Figs. 1 and 2. Transition from work to rest.

× c.c. of oxygen absorbed per minute

● c.c. of oxygen absorbed per litre of blood

■ Resting level.

Fig. 1. C.S.T. Age 29. The amounts during work are the average of six determinations; those during rest the average of seven determinations.

The following numbers were obtained in the successive determinations in the transitional period:

Ventilation in litres per minute: 20.5, 18.3, 18.2, 16.9, 11.6, 11.9, 9.9, 10.0, 9.0, 9.3, 19.0, 8.5, 8.6.

O<sub>2</sub> c.c. per minute: 876, 824, 844, 667, 380, 340, 314, 329, 300, 287, 305, 256, 266.

R.Q.: .09, .88, .88, .01, .02, .08, .02, .84, .82, .83, .96, .87, .81.

O<sub>2</sub> c.c. per litre blood: 98.5, 193, 64, 65, 61, 71, 59, 54, 63, 70, 73, 59, 66, 65.5, 64.5.

Pulse rate given in Fig. 3.

Fig. 2. E.H. Ago 28. The amounts during work are the averages of four-six determinations; those during rest the averages of four determinations.

The following numbers were obtained in the successive determinations in the transitional period:

Ventilation in litres per minute: 29.8, 19.3, 18.9, 21.9, 16.7, 18.35, 16.2, 19.8, 11.1, 11.8, 19.2.

O<sub>2</sub> c.c. per minute: 074, 866, 405, 436, 352, 374, 327, 294, 290, 325, 266.

R.Q.: .93, .94, 1.32, 1.56, 1.29, 1.29, 1.15, 1.14, .98, 1.12, .93.

O<sub>2</sub> c.c. per litre blood: 123, 115, 74, 70, 68, 64, 80, 77, 72, 61, 63, 79.5, 58, 68.

Pulse rate given in Fig. 3.

results are arranged according to the time elapsed from cessation of work to the middle of the experiment; the first is begun immediately after stop and has lasted a minute, the following experiments have lasted somewhat longer and the latest, as the resting experiments, some four minutes. The oxygen consumption as determined in this way is owing to the "dead space" in the Douglas bags in the work and after work experiments a little (some 2 p.c.) too low. More serious errors may, however, be caused by the subjects. It is a necessary condition for obtaining a reliable result, that the volume and the composition of the alveolar air remain unaltered during the experiment, especially when the experiment is short. If the alveolar air is altered, errors amounting to about 10 p.c. of the value are possible (Lindhard (20)). The composition of the alveolar air has not been determined in the present experiments but the mutual agreement between the two curves and their agreement with the results of previous experiments performed in a quite different manner make it probable, that significant errors due to variations in the composition of the alveolar air are not present. In the case C.S.T. the two last oxygen figures are some 10 p.c. lower than are the resting values. It is most probable, that this difference is real and owing to the fact, that the resting experiments were performed five-six weeks



before the after work experiments; the subject was at that time untrained in respiration experiments and felt tired from the long sitting on the ergometer.

In both cases the lung ventilation decreases evenly, most regularly in C.S.T. The respiratory quotient behaves on the whole in a manner similar to that described in the previous paper of Krogh and Lindhard(19). In E.H. the rise in the quotient within the first minutes after work is very pronounced and rather long lasting while in C.S.T. the rise is only slight. C.S.T. was a very strong and well-trained man while E.H. at that time was quite untrained.

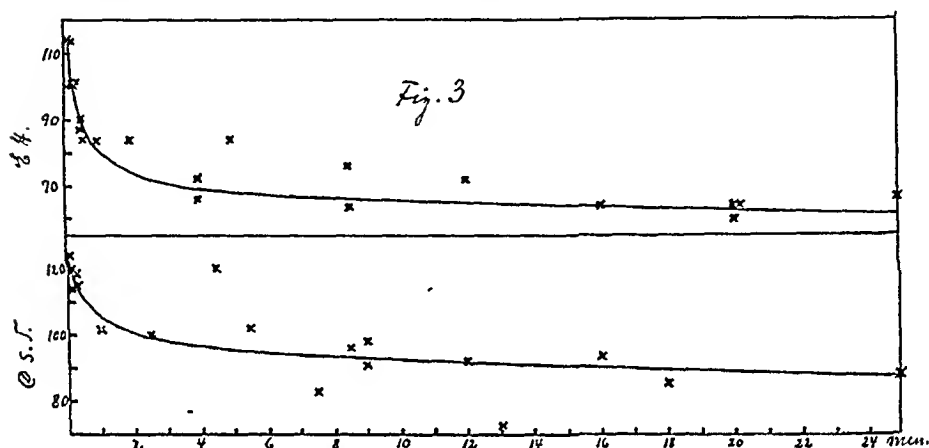
The circulatory function directly determined is the *utilization*, the oxygen absorption per litre blood, calculated in the manner described above. The utilization during work is in both subjects the double of the resting value and agrees fairly well with the values found by Lindhard(14) in other subjects working at a corresponding rate. *In the first few seconds after work has ceased we find a very slight decrease in utilization (Figs. 1 and 2, middle curves) followed by a rather rapid decrease for about two minutes. Three to four minutes after cessation of work the utilization in both subjects has gained the resting level but the decrease is then followed by an increase to a relative maximum at seven to eight minutes after work when the oxygen absorption is 70 resp. 80 c.c. per litre blood. Then we observe a new minimum followed by a maximum after 12 and 16 minutes respectively. From the last slight maximum the curve seems to run nearly straight and to attain the resting level 20 to 30 minutes after cessation of work. In the case C.S.T. the curve has been drawn by the aid of a later experiment falling without the frame of the figure. One of the experiments with E.H. at 16 minutes gives undoubtedly for unknown reasons too high a result. The curve has been drawn without regard to this experiment. The shape of the two curves is rather peculiar but the mutual agreement of the curves and the fact, that experiments performed at the same time after work with an interval of several days as a rule have given very nearly the same result make it very probable, that the shape of the curves is correct.*

It is worth noting, that experiments after static work have given a quite different result (Lindhard(20)). When static work ceases the utilization of the oxygen in the blood increases more or less from the working level which always lies lower than at rest and then rapidly falls down again to values below the resting level which seems to be regained within about five minutes.

The utilization cannot, however, be regarded as the essential circulatory function but it must depend on the metabolism and the circulation rate. It must be concluded from the observations and calculations of Krogh<sup>(21)</sup>, that circulation is not the limiting factor for the oxygen supply of the organism; the circulation must be regulated with regard to other substances or the minute volume may depend solely on mechanical factors: variations in the peripheral resistances, venous pressure and mechanics of respiration. At rest the utilization is rather small and of fairly constant magnitude, indicating, that an oxygen reserve in the circulating blood is always wanted to meet suddenly increased call for oxygen from the working organs. When working the utilization may be regarded as an index of the more or less suitable adaptation of the circulation to the amount of work done. The independent variables in circulation are the minute volume and the pulse rate, yet it must be remembered, that the former may in extreme cases depend upon the latter. The *minute volume* cannot, however, be determined directly in living men; in the present paper it is calculated from the oxygen consumption and the utilization (Figs. 1 and 2, upper curves). Apart from the first one or two minutes after cessation of work when the curve like those for the utilization and oxygen consumption presents a steep fall, the curve for the minute volume is symmetrical to that for the utilization, that is to say, at about two minutes after work the slope of the curve becomes less steep, in the case C.S.T. it runs nearly horizontal for three minutes, and gains at seven-nine minutes a minimum, where the minute volume reaches the resting level; it then rises again to a maximum at about 12 minutes which is followed by a minimum at 16-17 minutes. From this point the curve after a slight rise goes straight to the resting level. The waving course of the curve, which, according to what is said above, must be regarded as real, cannot have any relation to mechanics of respiration and the cause may therefore be sought in a periodic decrease and increase of peripheral resistances due to a simultaneous periodicity in the action of the vasomotor centre. Each period seems, especially evident in the case C.S.T., to be very nearly four minutes. No explanation of this periodicity can, however, be given at present.

The determination of the *pulse rate* is less accurate than that of the other functions dealt with. The pulse rate is counted in the usual clinical manner on the radialis or in C.S.T. often (during work always) on the carotis. In the first two minutes after work the pulse has only been counted for some 10 seconds. This method may involve a proportionally great error in the results given in number of beats per minute

but as the rate changes rapidly it was considered necessary to confine the observations to short time intervals. During work countings over longer periods are very difficult to obtain without missing one or more beats owing to unconscious twitches in the muscles. Moreover it must be borne in mind, that the pulse rate is a rather labile function, the normal rate being often disturbed by accelerating nervous influences. The results of the pulse countings are plotted in the curves, Fig. 3.



Like the other functions figured the pulse rate decreases rapidly when work ceases for one or two minutes and especially in the first half minute. In the table below are given the results of two countings begun three seconds after stop. The counting was continuous and each 10 secs. was marked. The figures in the table indicate the rate at 8, 18, 28 and 38 secs. after stop.

E.H. pulse rate after work:

9/3	114	102	90	84
15/3	114	102	87	—

After the initial steep fall the pulse rate decreases evenly and slowly to the habitual resting level. In C.S.T., whose pulse rate at rest is high, the observed figures are spread evenly above and below the curve. The observations at  $4\frac{1}{2}$  and 13 minutes deviate widely, especially the last-named figure is very difficult to explain. In E.H., whose habitual pulse is rather slow, the curve might seem to be drawn somewhat arbitrarily; if we consider, however, the nine observations within the first minute, the two at four minutes and the three at 20 minutes, it must be admitted, that the curve chosen is the most probable one and, that the deviations from the curve may be explained as due to nervous influences.

From the curves representing the minute volume and the pulse rate the output *per beat* of the heart can be calculated and, owing to the straight course of the pulse curve, the curve of the output per beat must run parallel to that of the minute volume. When the pulse rate has been observed in immediate connection with the circulation experiment the output per beat may be calculated from the two corresponding figures; as, however, the pulse rate independent of the minute volume may be disturbed by nervous influences it is possible, that the results calculated in this manner do not represent the really existing output per beat. Apart from this source of error it is obvious, that all uncertainties on the whole experiment arising from the determination of the oxygen consumption per minute and the utilization as well as from the pulse counting may accumulate on the figure representing the output per beat. Figures to be calculated from the curves will show, that the output per beat decreases rapidly and gains an absolute minimum at seven to nine minutes after stop corresponding to the first minimum of the minute volume. In both cases the output per beat during work is nearly twice the resting value.

#### SUMMARY.

The calculation of circulation experiments according to the nitrous oxide method is simplified.

The direct result of the calculation is the oxygen absorption per litre blood. From this figure and the oxygen consumption per minute as determined in a respiration experiment the minute volume of the heart is calculated.

The results of experiments dealing with the oxygen consumption per minute, the oxygen absorption per litre blood and the pulse rate within the first 20 minutes after cessation of muscular work in two male subjects are given.

The minute volume of the heart as calculated from the experimental data shows that the circulation rate decreases rapidly during the first two minutes and then more slowly following a rather complicated curve.

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## SEASONAL VARIATION IN THE RETICULATED CORPUSCLES OF AMPHIBIAN BLOOD.

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(From the Physiological Laboratory, Cambridge.)

*Methods.* A reticulated appearance in the cytoplasm of the red blood corpuscle of the frog<sup>1</sup> (*Rana temporaria*) and the toad<sup>1</sup> (*Bufo vulgaris*) is very well and easily displayed by the following method. A specimen tube (size 5 cm.  $\times$  1.2 cm.) is three-quarters filled with .1 p.c. methylene blue in frog's Ringer. The animal being anaesthetised with .5 c.c. saturated solution of urethane its heart is exposed; a small clip is fastened on the apex to weight it; the arterial arches are cut across; the frog is turned belly down and the blood is allowed to flow into the methylene-blue-Ringer. The corpuscles are distributed throughout the fluid by occasionally inverting the tube. After an interval of 1-2 hours a drop of the fluid is examined under a  $\frac{1}{17}$ th inch oil immersion. At this time the reticulum is usually distinct, though it may go on developing in distinctness for several hours more.

*Appearance of a reticulated cell in methylene-blue-Ringer.* The appearance of a typical cell with well developed reticulum may be taken first. The nucleus is always well-stained, varying in shade from light to dark blue, but it is sometimes violet and then stains very deeply. The chromatin is well seen. The cytoplasm is of a greenish hue. The reticulum when well developed extends throughout the cytoplasm. It is not on the same focussing level throughout but is of course on much the same level as the equator of the nucleus. It may consist either of a series of fine closely set granular dots or a fine close fibrillar network, or it may consist of fewer comparatively coarse fibres forming a more open network. It varies considerably. It may be small in amount and faint or profuse and faint; it may be slight in amount and definite or profuse in amount and definite. It may be absent. The reticulated appearance is best seen in fresh preparations. The only method of fixation which does not destroy it is with 1 p.c. picric acid of ammonia in frog's Ringer.

In using the expression reticulum it is desirable to refrain from any

<sup>1</sup> The animals used were examined within a few days after collection from the fens.

implication as to its nature. It may be that the reticulum is not existent as such in the cell *in vivo*, but none the less, even if it is a post mortem artefact, would its production in only a proportion of cells and in a different proportion at different seasons be significant of some precursor substance or state in the cell. Nor does the use of the word reticulum imply that it is necessarily rigid. It certainly does not hold the nucleus in position, for the nucleus may sometimes be observed displaced or sometimes in an oblique position in reticulated cells.

Definite large granules are often seen in the cytoplasm. These may number from one to nine or ten. They may occur in cells with or without a reticulum, but if they occur in reticulated cells the reticulum is usually coarse and scanty. They vary in size from being almost invisible to large coarse granules as big as the granule of the human eosinophile cell. They may occur in clumps or scattered singly throughout the cell. They may occur in any position in the cell and if they overlie the nucleus they are not on the same focal plane as it. They may occur only in a few cells or in all the cells. In colour they are sometimes deep blue, often violet and occasionally show a distinct pink tinge. They often increase indefinitely in size from hour to hour so long as they are under observation.

*The degeneration which occurs in some cells in methylene-blue-Ringer.* This is observed in the cytoplasm in a varying number of cells after an interval which varies in different animals and at different times of the year. It was observed first in marked degree in February. Attention was then called to it by the fact that blood run into methylene-blue-Ringer at midday showed degeneration in numbers of cells by 3 p.m., necessitating careful watch over the process of staining so as to get the optimum moment for doing the differential count. It continued to be a marked feature of the blood picture till June. It did not interfere with the differential count during the rest of the year even when 4-6 hours were allowed to elapse before it was made, *i.e.* it certainly did not occur within the first 6 hours and, on every occasion on which attention was directed to this point not to any marked extent in 24 hours, unless the animal was very poorly nourished.

It usually occurs in unreticulated cells but may occur in reticulated. It is not affected by the strength of methylene-blue and is observed also in plain Ringer. In spring it renders the distinction of reticulated from unreticulated cells difficult. When it occurs the cytoplasm of the cell becomes granularly refractile or takes on a ground glass appearance. An edge of the cell may fold over or the cell membrane may show transverse lines suggesting incomplete fractures. Finally, the cell disintegrates

and the nucleus appears free. In the early stages of this process the degenerating cell is further characterised by the fact that its nucleus takes the stain comparatively poorly and sometimes not at all.

*Differences between frog and toad.* These appearances—reticulum, metachromatic granules and degeneration—are observed in the cells of both the frog and the toad and no great differences are observed between them in these respects, except that on the whole degeneration is more common in frogs. This is attributed to the fact that the year during which these observations were carried out was a good one for toads but bad for frogs and the frogs were in a poorer state of nutrition than the toads.

*Seasonal variation in the proportion of reticulated cells.* If a specimen of blood be examined in July or August one is struck by the large proportion of reticulated cells present. Reticulated cells are usually acknowledged as being young cells and one at once associates their presence in such large proportion at this season with the large production of new cells which is believed to occur in late spring and early summer (Ecker). The questions then arise—if the new blood cells are formed largely at this season does the proportion of reticulated cells rise in summer and fall towards winter and secondly does the reticulum undergo any change in appearance with the ageing of the cell as the season advances? These questions were approached by examining the blood from animals killed at intervals throughout the year, counting the proportion of reticulated and unreticulated cells and examining the character of the reticulum at different seasons.

Detailed results are given below in the table, but the results may be anticipated here to this extent: we may say that the reticulum in amphibians is much more persistent than the reticulum in the cells of mammalian blood. The latter is a rarity, the former is the rule; in the amphibian the reticulum persists in some form for the greater part of the life of the cell. Its disappearance cannot be said to herald the destruction of the cell. It usually does so, but in poorly nourished frogs, *i.e.* frogs which have no food material in their stomachs and rectum, there may be no spring formation of red cells and the unreticulated cells then persist throughout the summer. The ageing of the cell, in so far at least as it is indicated by the disappearance of the reticulum, is not at any rate the sole cause of destruction of the cell. That destruction is due to unknown factors of which one may be these changes in the cell and another possibly the activity of the blood destroying organs.

In interpreting the table we must remember that we are dealing



with widely varying figures due to the mixing of animals of all ages collected in different localities and under different conditions of nourishment, but we may say that reticulated cells exist in amphibian blood in high proportion in late summer and in reduced numbers throughout the winter till spring and then disappear. The proportion of unreticulated cells grows steadily at their expense, to suddenly fall next summer when the new crop of reticulated cells makes its appearance. The time of appearance of the new crop is somewhat dependent on the character of the season and the abundance of the food supply. It is to be noted that the spring during which these observations were carried out (1922) was on the whole cold.

*Variations in appearance of reticulum according to age of cell.* In the typical cell the reticulum is described as either of finely granular appearance or of fine or coarse fibrillar appearance. These may be seen at one and the same time in the same frog. The first two are, however, common when the fresh crop of reticulated cells first appears; the last appearance is common in late autumn; so that these probably represent successive stages in the development of the reticulum. However this may be, there is no doubt that in winter the reticulum is slightly coarser and much smaller in amount, so that it may be represented only by a few coarse threads scattered here and there in the cytoplasm. In early winter all stages may be observed between the fully developed coarse reticular network and its complete disappearance. Incidentally it may be remarked that this adds to the difficulties of counting the percentage of reticulateds, as including all the cells with only traces of reticulum amongst reticulated cells gives a wrong statistical impression of the appearance of the blood. Thus the change which occurs in March from a moderate to a low percentage of reticulateds would appear from the tables to be more abrupt than it actually is, because just previously the cells counted as reticulated are showing each only a small amount of reticulum.

In watching the changes in the blood cell throughout the seasons one is forced to the conclusion that on the whole the great majority of them pass through a complete cycle in the course of the year and, in the average healthy frog or toad in a normal year, are almost completely replaced each spring. This rule, like all rules, is liable to exceptions.

TABLE SHOWING PERCENTAGE OF RETICULATED CELLS IN BLOOD.

The observations marked \* were made in 1922; the others in 1921.

Each number represents the count on a different animal made during the month.

Month	Frog	Toad
I. *June 15-30	01, 53, 10, 43, 32, 100, 67, 88, 52, 78, 51	80, 45, 14, 7, 0, 0, 71, 98, 53, 46, 63, 49
*July	23, 67, 65, 72, 0, 17	72, 57
July	100, 00, 21, 17, 03, 14, 19	—
*Aug.	0, 85	47
Aug.	86, 96, 95	—
Oct.	83, 04	—
Average	56 %	46 %
II. Nov.	33	46, 40, 47
Dec.	—	67, 53, 22
Jan.	14, 47	0, 25, 0
Feb.	—	15, 31
Mar. 1-15	73, 80, 85, 0, 45, 32, 62	45, 30, 32
Average	47 %	32 %
III. Mar. 16-31	1, 1	11, 8, 13, 21, 7
Apr.	0	17, 3, 0, 0, 0
May	0, 0, 0, 0, 0, 0, 0, 0	4, 3, 2
June 1-15	0, 0, 0	0
Average	0	6 %

### CONCLUSIONS.

The blood of the frog and of the toad shows a high proportion of reticulated cells in late summer. This high proportion persists for some time, though the reticulum changes in appearance and becomes smaller in amount. In late spring and early summer, prior to the appearance of the new crop of reticulated cells, the proportion of reticulated cells falls to a minimum. At this period too the cells degenerate quickly in 1-1000 methylene-blue-Ringer. The majority of the red cells of the frog and the toad pass through their complete cycle in the course of a year.

# A STUDY OF THE CHLORINE INTERCHANGE BETWEEN CORPUSCLES AND PLASMA.

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IN 1861 Zuntz(1) showed that after blood is saturated with carbon dioxide the plasma becomes capable of taking up more carbon dioxide in combination than is the case if it be separated from the corpuscles before saturation. He concluded that in the presence of an increased partial pressure of carbon dioxide alkali passes out from the corpuscles into the plasma. Later Gürber(2) showed that what really happens is that hydrochloric acid passes into the corpuscles, thus leaving the plasma more alkaline, and therefore more capable of taking up carbon dioxide. Hamburger(3) obtained a similar result, but interpreted it as due to the passage of chlorine ions into the corpuscles, and further showed that when carbon dioxide is removed from blood the reverse reactions take place. Parallel with this phenomenon the corpuscles increase in diameter as may be shown by a corresponding increase of corpuscular volume by means of the hæmatocrit. More recently de Boer(4) and Hamburger(5) have shown that a similar transference of  $\text{SO}_4$  ions occurs on the addition to blood of carbon dioxide and of hydrochloric acid. Van Slyke and Cullen(6) and Fridericia(7) have recently described series of careful experiments and have confirmed the essential points of the phenomenon, viz. the passage of chlorine ions through the corpuscular membrane under the influence of carbon dioxide.

The experiments described in the following pages were undertaken for the purpose of confirming some of the previous conclusions with regard to carbon dioxide and in addition of ascertaining the influence of "acidosis" produced in various ways upon the passage of chlorine into the corpuscles. It is this last question which has principally engaged our attention. Under normal conditions of the circulation it seems probable that the reduction of hæmoglobin is the most important factor in minimising variations of pH when arterial blood becomes venous. The transference of chlorine ions caused by changes in carbon dioxide tension within physiological limits must be very small. It seemed to us to be important to determine whether any such transference may occur in conditions of "acidosis."

The experiments were carried out in the Department of Therapeutics, Edinburgh University. Our thanks are due to Prof. Meakins and Mr C. R. Harington for assistance and advice, and to Dr J. S. Haldane for helpful criticism of our results.

*Methods.* In all the experiments normal human blood was used, clotting being prevented by the addition of a constant small amount of neutral potassium oxalate (0.1 p.c.). In all the experiments with carbon dioxide and with other acids, the blood was fully oxygenated. A few c.c. of whole blood were reserved for estimation of total chlorides, the remainder within a few minutes of being drawn was placed in a saturator similar to that used by Christiansen, Douglas and Haldane (8). Carbon dioxide or other acid was added and the saturator was then rotated in a water bath at 37° C., the excess of pressure being released after the first five minutes. After 15 minutes the saturator was removed from the bath and quickly wrapped in warm cloths. Samples of blood were then taken for estimation of carbon dioxide by the method of Haldane (9), duplicate determinations agreeing to within 0.5 volume p.c. A sample of air from the saturator was also analysed with the small type of Haldane gas analysis apparatus. In addition two samples of blood each of 10 c.c. were placed under paraffin in graduated centrifuge tubes. The blood was then centrifuged at 33° C. for 45 to 60 minutes, 45 minutes having been found sufficient for complete separation, after which the relative volume of plasma and cells was read off and reduced to percentage. Chlorides in plasma and cells were then immediately estimated separately and in duplicate by the method of Wetmore (10), the proteins being precipitated by copper hydroxide, phosphates and oxalates being subsequently removed by shaking the filtrate with a small quantity of calcium hydroxide and refiltering. The few experiments where the difference was of more than 10 mg. (*i.e.* 2 p.c.) in duplicate determinations were eliminated. *pH* was not measured directly but calculated from the formula of Hasselbalch:  $pH = pK + \log \frac{NaHCO_3}{H_2CO_3}$  taking 6.1 as the value of *pK*.

*Experiments with carbon dioxide.* Fridericia (7) has shown that for samples of blood taken from the same animal at the same time and exposed to varying pressures of carbon dioxide, the chlorides of plasma vary in inverse ratio to the pressure of carbon dioxide. The curve so constructed could be superposed upon the dissociation curve of carbon dioxide in blood. In our endeavours to extend these observations to human blood at 37° C. we found it impossible to obtain a consistent curve for plasma chlorides at varying carbon dioxide pressures when

experiments were performed on different days. The results when plotted fell only very approximately on the theoretical curve. The reason for this can readily be seen from the tables, which show that the amount of total chlorides in blood varies in different people and in the same individual from day to day, even in spite of the fact that in our experiments the blood was always taken at the same time of the day (between 11 a.m. and 12 noon). It appears however that when the partial pressure of carbon dioxide increased above 150 mm. no further transference occurred.

The carbon dioxide dissociation curve of H. W. D. has already been published (16). The points obtained for the blood of H. W. D. in the present experiments fall exactly upon this curve. This fact is of some

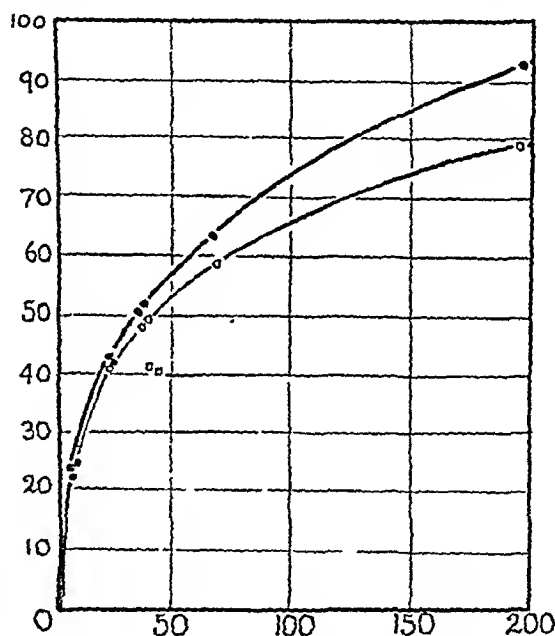


Fig. 1. Carbon dioxide dissociation curve for fully oxygenated whole blood of L. D. Upper curve—total carbon dioxide. Lower curve—combined carbon dioxide. Abscissa—pressure of carbon dioxide (mm. of Hg). Ordinate—c.c. vols. p.c. of carbon dioxide. Points marked □ are for blood after exercise of H. W. D.

interest, as the curve of H. W. D. was determined some eighteen months previously, since when he has travelled round the world, experiencing great variations of climate. A complete carbon dioxide dissociation curve for the blood of L. D. is given (Fig. 1). This agrees almost exactly with the curve of H. W. D. and with the original curve for Haldane (8). We have always found that the corpuscular volume as shown by hæma-

to crit readings increased with increasing tensions of carbon dioxide. However in our experiments where other acids were added to blood the hæmatocrit readings were somewhat irregular. Table I shows results obtained on exposing blood from three different subjects to varying partial pressures of carbon dioxide. These results will be further discussed below.

TABLE I. Blood exposed to varying partial pressures of CO<sub>2</sub>

Subject	CO <sub>2</sub> pressure mm. Hg	$\text{pH} = \text{pK}_1 + \log \frac{\text{NaHCO}_3}{\text{H}_2\text{CO}_3}$ $\text{pK}_1 = 6.1$	Vol % of CO <sub>2</sub>		Hæma tocrit. Cells %	Chlorides mg %		Total chlorides mg %	% of total chlorides in plasma	O <sub>2</sub> saturation of bl
			count	Normal (Haldane)		Cells	Plasma			
L D	—	—	55.02	—	39.8	—	579	480	72.6	Venous
"	35.2	7.40	48.3	48.5	33.5	—	603	480	83.5	100 p.c.
W.A.	32.01	7.00	31.2*	50	35.7	321	—	514	—	"
"	315	7.14	27.9*	48	35.4	302	—	509	—	"
H.W.D.	42.5	7.33	51.6	52	36.7	318	662	519	80.7	"
"	143.3	6.96	78.8	80	37.3	360	587	519	70.9	"
L.D.	113	7.65	35.5	33.5	37	275	620	483	81.9	"
"	671.0	6.41	140.0	—	13.3	315	557	189	75.8	"
L.D.	68	7.80	23	24	36.6	258	663	510	82.4	"
"	195.0	6.87	92.3	—	39.3	326	600	510	73.3	"

\*Normal acidosis. Growing boy (see Schloss, *Amer J Dis of Child*, 13, p 210)

TABLE II. Blood at different degrees of oxygen saturation.

L D	24.3	7.50	13.2	42	40.6	294	593	468	73.1	19 %
"	24.3	7.50	43	42	40.8	271	591	462	73.9	100 %
L.D.	32.1	7.17	52.7	47	31.5	289	608	512	77.7	Reduced blood
"	38.6	7.37	51.6	50.0	33.1	280	607	500	81.2	100 %
H.W.D.	37.9	7.45	51.97	49.8	—	284	578	460	—	0 %
"	43.0	7.30	50.98	52.1	—	282	575	—	—	100 %
H.W.D.	22.6	7.54	14.57	40.5	33.1	235	481	102	78.6	1 %
"	28.4	7.42	11.07	41.4	35.6	232	490	102	79.3	100 %

TABLE III. Addition of acid to blood

L.D.	36.8	7.25	38.1	49.5	27.4	—	579	512	82.1	0.3 c.c. lactic acid
"	36.1	7.39	50	49	28.1	—	581	512	81.5	Normal
H.W.D.	7.6	7.57	15.5	25.5	35.2	329	631	509	80.1	0.3 c.c. lactic acid
"	7.6	7.79	25.8	25.5	33	335	631	507	83.7	Normal
"	2.7	—	0	10	36.3	423	620	520	75.9	1.3 c.c. lactic acid
"	6.2	7.85	21	23	30.3	309	689	527	83.2	Normal
L.D.	79.3	6.97	45	66	38.6	364	605	515	72.1	0.5 c.c. oxybutyric
"	67.5	7.21	63	62.5	38.5	341	625	515	74.6	Normal
H.W.D.	0.91	—	0	9	36.6	399	612	536	72.3	1.5 c.c. oxybutyric
"	2.71	8.06	16.9	16	35.1	267	675	536	81.7	Normal

TABLE IV. Severe exercise

H.W.D.	10.4	7.29	52	53.5	32.4	305	633	525	81.5	Normal
"	41.5	7.21	41.9	52	31.9	344	639	545	79.8	After severe exercise
"	48.3	7.27	54	54	35.1	320	612	499	79.6	Normal Hb 96 %
"	43.0	7.21	40	52.5	38.1	313	615	500	76.1	After severe exercise Hb 103 %

Each pair of observations, 1 a, 1 b, etc., were made on the same day, using different portions of the same sample of blood except in Table IV

*Observations with reduced hæmoglobin.* Christiansen, Douglas and Haldane (8) have shown that oxyhæmoglobin behaves as if it were more

acid than reduced hæmoglobin. We have endeavoured to ascertain whether this property has any influence on the transference of chlorine from plasma to corpuscles or *vice versa*. The blood in the saturator was exposed to a current of hydrogen freed from acid by being first passed through a wash bottle containing caustic soda. In order to accelerate reduction, the saturator was placed in the bath at 37°. Five to ten minutes were sufficient for almost complete reduction. As can be seen from Table II, blood with hæmoglobin reduced to 2 p.c. of oxygen saturation and then exposed to carbon dioxide has a pH (calculated) of 7.47 while with fully oxygenated hæmoglobin the pH is 7.37. In spite of a difference of carbon dioxide pressure in the experiments 3 and 4 of 6.5 mm. of Hg in favour of the oxygenated blood, it still took up less carbon dioxide than the reduced blood. The calculated pH changes from 7.37 to 7.47, yet there is no difference in the chlorine percentage of the plasma. These results were confirmed later in experiments 5 to 8. In spite of increased carbon dioxide combining power in reduced blood, the chlorine transference does not take place. This seems to indicate that in the living body, where the oxygen desaturation of mixed venous blood is about 30–40 p.c., and the increased carbon dioxide pressure about 8 mm. of Hg, the interchange of chlorine when arterial blood becomes venous would be very small, scarcely beyond the limits of experimental error. Our results for carbon dioxide content and calculated pH given in Table II fall exactly upon the curves given by Parsons(12). Exps. 6 *a* and 6 *b* are interesting in that there appears no increase of carbon dioxide capacity when the hæmoglobin is reduced. This result accords with those of Henderson and Haggard(17), which have been discussed by Van Slyke(18) and also by Douglas and Haldane(19). We attribute this result to delay (34 minutes) while the blood was being reduced and consequent lactic acid formation facilitated probably by the low partial pressure of carbon dioxide as shown by Lovatt Evans(20). Our later results where precautions were taken against delay show results which accord with those of Christiansen, Douglas and Haldane(8).

*Acids other than carbon dioxide.* (1) Experiments with lactic acid were performed in two manners—firstly by the addition of lactic acid to blood *in vitro* and secondly by the production of lactic acid *in vivo* by severe muscular exercise. Ryffel(13) has shown that blood after severe exercise may contain .07 p.c. of lactic acid, so for an experiment *in vitro*, to 25 c.c. of blood we added .03 c.c. of 9.7 N lactic acid in order to obtain an increase of hydrogen ion concentration without exceeding the physiological limits. In Exp. 10 we added this amount of acid, firstly with

blood exposed to partial pressures of carbon dioxide within physiological limits and afterwards (Exp. 11) with a very low carbon dioxide pressure, but sufficient however to leave some of the "bicarbonate reserve" intact. In none of these experiments were we able to detect any chlorine transference between plasma and corpuscles. In Exp. 10 the carbon dioxide capacity is diminished by 23 p.e. which agrees sufficiently with the value of 24 p.c. given by Mellanby and Thomas(15) for the same quantity of acid. In Exp. 12 it is diminished by 39 p.e. It is noteworthy that in Exp. 12 where the quantity of acid was the same as in Exp. 10 the fall of carbon dioxide combining power is less (10 vols. p.e. instead of 11.5 below the normal curve).

Van Slyke and Cullen(6) have shown that when the acid added to plasma corresponded with about half of the bicarbonate present, the fall of "alkaline reserve" corresponded approximately in molecular equivalents to the quantity of acid added. If the acid increases the fall of bicarbonate is not corresponding. In Exp. 13 *a* we added a quantity of acid sufficient to neutralize all the bicarbonate; in this case there was a considerable transference of chlorine.

Hamburger(5) has shown that an interchange of chlorine ions was produced on the addition of acid to the blood. One must remark, however, that he used far greater quantities of acid than were used in our experiments; and, just as in our Exp. 13 *a* the whole of the available alkali must have been neutralised.

When introducing strong acids to blood in order to prevent any precipitation on the sides of the saturator or other gross damage to corpuscles, our technique was as follows: The blood was introduced by means of a 25 c.c. pipette into the bottom of the saturator which was held vertically, the acid having been previously spread as diffusely as possible on the upper part of the sides. The saturator was then corked and the whole contents suddenly and violently agitated. By this method it was possible to avoid obvious damage to cells by the strong acid. When the blood was subsequently centrifuged, no hæmolysis and no layer of precipitated proteins at the bottom of the tubes were observed. The general technique of our experiments with lactic acid (0.13 c.c. of 9.7 normal acid with 25 c.c. of blood) as well as with  $\beta$ -oxybutyric acid (0.15 c.c. 7.6 normal acid for 25 c.c. of blood) differed slightly in some cases from our usual technique described at the beginning of this paper in that the air of the saturator, after the addition of the acid, was as far as possible freed from carbon dioxide by means of a current of air sucked through by a filter pump. The saturator was placed in the bath for five mins. after which it was taken out and again connected with this filter pump in order to remove any further carbon dioxide liberated from the blood by the acid. After this the procedure was the same as described above. By this means the blood could be equilibrated with an atmosphere almost completely free of carbon dioxide.

As regards muscular exercise two experiments (Table IV) were undertaken, both on the same subject (H. W. D.) and differing only as regards



the duration of the exercise. The first consisted in pedalling on the Martin bicycle ergometer for an hour at the rate of 592 kilogram-metres per minute. At the end of each quarter of an hour the work was increased to 1576 kg.-metres per minute for 5 mins. After the exercise the subject experienced great exhaustion accompanied by nausea and dizziness. The second experiment was more complete, samples of urine being collected before and after the exercise, and the alveolar air being estimated. The duration was less and the exercise more violent, the object being to obtain a maximum acidosis by minimising the compensatory processes which probably occur with less severe exercise over a longer period. So as can be seen in the following protocol the acidosis was greater.

Subject H. W. D. Barometer 753.

11 a.m.	Resting. 11.15. Bladder emptied.
11.30	Blood drawn—at 48.3 mm. CO <sub>2</sub> pressure, blood took up 54 vols. p.c. of CO <sub>2</sub> . (Normal 54 ∴ alkaline reserve ± 0.)
12.30 p.m.	Resting on bicycle ergometer.
12.45	Alveolar air CO <sub>2</sub> 5.87 p.c. = 41.4 mm. CO <sub>2</sub> pressure.
12.47	Urine collected. 11.15 a.m. to 12.47 p.m. 370 c.c. containing 14 % N/10 alkali. Ammonia nitrogen 0.0 mg. %. Urea nitrogen 224 mg. %. Ammonia: urea nitrogen ratio 0. NaCl 635 mg. %. Albumen 0. Acetone bodies 0.
12.48	Pulse 70. Respirations 13.
12.50	Commenced pedalling on ergometer. Rate of work 982 kg.-metres per min. Pulse 111. Resp. 25.
12.52	982 kg. metres per min. Pulse 112. Resp. 26
12.54	982 " " " 110. " 26.
12.56	982 " " " 110. " 26.
12.58	Resting. Pulse 120. Abundant perspiration.
12.59	" " 100. Resp. 19.
1.0-1.2	1629 kg.-metres per min. Maximum possible effort.
1.2	Work stopped. Pulse 120. Exhausted. 1.3. Pulse 100. 1.4. Pulse 88. 1.5. Pulse 100.
1.7	Alveolar air CO <sub>2</sub> 5.70 % = 40.2 mm. CO <sub>2</sub> pressure.
1.8	" " 4.94 % = 34.9 " " "
1.10	Blood drawn. At 43 mm. CO <sub>2</sub> pressure blood took up 40 vols. p.c. of CO <sub>2</sub> . (Normal 52.5 ∴ alkaline reserve - 22 %)*
1.15	Urine collected. 12.47-1.15 p.m. 54 c.c. containing 6.6 % N/10 acid. Ammonia nitrogen 19.6 mg. %. Urea nitrogen 246.4 mg. %. Ammonia: urea nitrogen ratio .079. NaCl 635 mg. %. Albumen 0. Acetone bodies 0.
2.15	Urine collected. 1.15-2.15 p.m. 58 c.c. containing 24 % N/10 acid. Ammonia nitrogen 50.6 mg. %. Urea nitrogen 243.4 mg. %. Ammonia: urea nitrogen ratio 0.207. NaCl 1073 mg. %. Albumen 0. Acetone bodies 0.

\* Further details in Table IV.

In the above experiment the titration of alkaline urine was done by the method previously described by Davies, Haldane and Kennaway (16). Acid urines were titrated to pH 7.43 in Cole's comparator using phenol red as indicator.

The percentage of chlorides in the cells (Table IV) is increased while that in the plasma remains unmodified. The analysis of the urines is in itself very instructive. Before commencing the experiment the urine was alkaline (a saline purgative having inadvertently been taken earlier

in the day) and the subsequent changes exhibited in a marked degree the production of acid, and the change in ammonia-urea ratio during heavy muscular work. The oliguria during and even one hour after the exercise was very striking. Also the urinary chlorides remained at exactly the same level as before the experiment and were increased only one hour afterwards when, as has been shown by Ryffel<sup>(13)</sup> and by Douglas and Haldane<sup>(14)</sup>, the lactic acid has been completely oxidised or eliminated. Unfortunately the quantity of urine was insufficient to allow of phosphate estimations being made.

(2) Experiments *in vitro* with  $\beta$  oxybutyric acid were carried out with exactly the same technique as with lactic acid, and the results were identical. We used 7.6 normal acid, kindly prepared for us by Mr Harrington. When it was added to the blood in the manner described above no destruction of blood cells occurred. The results (Exps 13 and 14) show that when all the "bicarbonate reserve" is not neutralised the transference of chlorides does not take place. In Exps 13 *a*, 13 *b* there is an increase of chlorides in the corpuscles and decrease in that of the plasma with acidified blood, but the carbon dioxide pressure is much higher and probably the chlorine ion interchange is due to this. The experiment when repeated with 0.15 c.c. of acid for 25 c.c. of blood, in order to neutralise all the bicarbonate, shows a considerable chlorine shift.

### DISCUSSION OF RESULTS

It is not necessary to discuss extensively the results for the experiments with carbon dioxide alone. Our work has merely confirmed the well-known results of previous workers, but is slightly more complete in that we have estimated the chlorine percentage in the corpuscular mass as well as in the plasma after centrifugation. We found it impossible to obtain a consistent curve for percentage chlorides in plasma or percentage of total chlorides in plasma with varying carbon dioxide pressure, or varying *pH*. This can partly be explained by the fact that the experiments were performed on different subjects on different days and with different percentages of chlorides in whole blood. The variation in total chlorides may be very large, thus in the case of H. W. D. there was a change of over 50 mg. on two consecutive days (Exps 8 and 9), while in Exp 15 after exercise his total chlorides were 545 mg. p.c. and in Exp 14 a year later they were 402 mg. p.c. At present we are unable to suggest any explanation for this variation. In the case of L. D. the maximum difference was 53 mg. These factors should be partly elimi-

nated by taking hæmatocrit readings and, knowing the percentage of NaCl in plasma and corpuscles respectively, calculating the percentage of total chlorides present in the plasma. Unfortunately our hæmatocrit readings were not always reliable—duplicate readings often failed to agree within 2 p.c. so that we lack accurate quantitative data for this calculation. It appears further that there may be some other factors involved. Possibly there may be changes in the corpuscular membrane as regards permeability to salts and to water, or variations in the hydrophilic and hydrophobic characters of the various colloids. All our experiments have shown that on a given sample of blood an increased partial pressure of carbon dioxide causes passage of chlorine from plasma to corpuscles but we have been unable to obtain consistent quantitative results similar to those of Fridericia who performed all his experiments on the one sample of blood.

For the other types of acidosis *in vitro* within physiological limits no measurable chlorine transference occurs, even when the bicarbonate reserve is greatly diminished. This effect comes into play only when all the bicarbonate has been neutralized. The following seems to us to be the most probable explanation. When any acid is added to the blood it attacks the alkaline salts of the proteins as well as the bicarbonate, the alkaline proteinate being the first attacked and the ratio  $\frac{H_2CO_3}{NaHCO_3}$  being maintained, at least with small quantities of acid. Only when the greater part of the alkaline proteinate is neutralized does the acid combine with the bicarbonate, and after that with the sodium of neutral salts, the latter being the most stable. This seems true as well for  $H_2CO_3$  as for stronger acids, the  $H_2CO_3$  having combined with all the Na of the proteins it cannot further combine with  $NaHCO_3$ , so that it then attacks the basic radicles of the neutral salts. This hypothesis is proved by the fact that on adding a little more than .12 p.c. of lactic acid to the blood (Exps. 10 *a*, 10 *b*) all the bicarbonate would be neutralized if the acid combined first with bicarbonate before attacking the Na proteinate. Instead of this being the case, the carbon dioxide content of blood treated in this way was only 10 and 11.4 vols. p.c. below normal, the carbon dioxide pressure being respectively 7.6 and 36.8 mm. of Hg. Moreover if we add .12 p.c. of lactic acid to a sample of blood at a very low pressure of carbon dioxide (Exps. 11 *a*, 11 *b*) and thus with a low bicarbonate content, the bicarbonate would be immediately neutralized if the fixed acid combined first with it. This is not so, for at these low carbon dioxide pressures the acidulated blood still contains relatively slightly more carbon dioxide than at higher carbon dioxide pressures.

That is, at low pressures of carbon dioxide the "bicarbonate reserve" is less diminished than at high carbon dioxide pressures on the addition of the same amount of fixed acid. This shows conclusively that the proteins are weaker acids than is  $\text{H}_2\text{CO}_3$ , and are the first to be turned out on the addition of a stronger acid. Although no chlorine transference occurs *in vitro* until all the bicarbonate reserve is neutralized, yet with an acidosis *in vivo* it is obvious that reduction of bicarbonate reserve would cause the transference to occur more readily when the blood becomes venous, just as in Exp. 10 b, because under such conditions the available alkali combined with proteins is diminished.

As regards our experiments with reduced hæmoglobin no further comment is necessary. They show definitely that at constant carbon dioxide pressure no chlorine shift occurs as a result of oxygenation or reduction of hæmoglobin.

The experiments in which severe exercise was performed show many interesting results. The absence of diminution of plasma chlorides is the same as occurs with small quantities of lactic acid added *in vitro*. The increased percentage of chlorides in the cells is a phenomenon which is difficult to explain. The following facts are certain:

(1) In the first experiment where the exercise was of long duration the chloride percentage of the whole blood was increased.

(2) The hæmoglobin percentage is increased, but the increase is greater than that of the chlorides. This is probably due to the increased capillary circulation washing out corpuscles from stagnant capillaries. It seems that, apart from their rôle in the regulation of blood alkalinity, the chlorides may also play a part in the regulation of osmotic pressure in the plasma, and may by some unknown means be retained in the corpuscles in order to maintain the normal osmotic pressure of the plasma. We are unable to offer any elucidation of the mechanism of this retention. Nolf(15) considered that in the maintenance of the normal osmotic tension of the plasma, the chlorine shift could be sufficiently accounted for by the varying carbon dioxide pressure, but our experiments appear to indicate that some further factor is involved. In our second experiment with exercise there was no increased chloride percentage in whole blood. This was due probably to the shorter duration of the exercise and smaller loss of water. The increased hæmoglobin would be due probably to the flushing out of the capillaries.

## SUMMARY.

1. Our results with carbon dioxide are in accordance with those of Zuntz, Gürber, Hamburger, Van Slyke and Cullen, Fridericia and other workers, except that under the conditions of our experiments we were unable to obtain quantitative results similar to those of Fridericia.

2. The carbon dioxide dissociation curve for one of us (L.D.) is published. Results for H. W.D. show that his dissociation curve already published remains unaltered.

3. With constant partial pressure of carbon dioxide, reduction of hæmoglobin produces no chlorine transference.

4. Acids other than carbonic acid whether added to the blood *in vitro* or produced *in vivo* do not of themselves cause any chlorine transference unless they be present in quantities sufficient to neutralize the whole of the "bicarbonate reserve."

5. Experiments with severe muscular work appear to indicate that chlorine transference may occur not only for the regulation of the blood alkalinity, but also possibly to maintain the normal osmotic pressure in the plasma.

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*Note.* In a Paper now in the Press (Heart) we have shown that with the extreme local carbon dioxide acidosis, the chlorine transference occurs *in vivo* in exactly the same manner as would be expected from the experiments *in vitro*.

# CALIBRATION OF THE REVERSION SPECTROSCOPE FOR THE ESTIMATION OF CO IN BLOOD.

By H HARTRIDGE

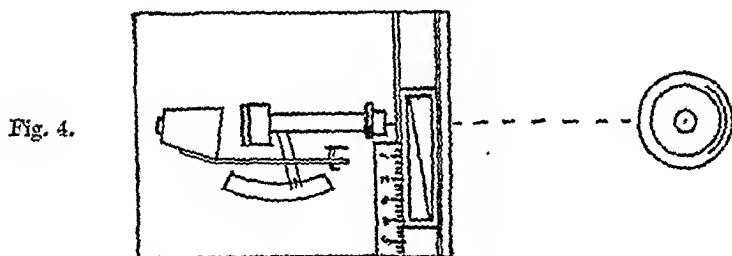
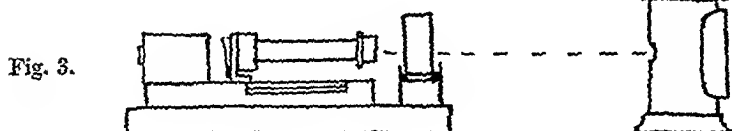
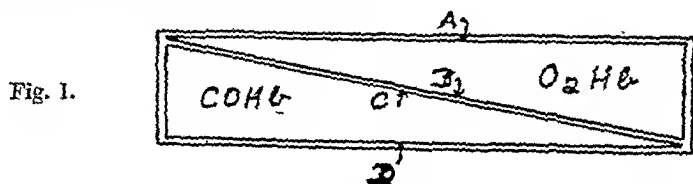
*(From the Physiological Laboratory, Cambridge)*

A SPECTROSCOPIC method of estimating the percentage saturation of blood pigment with carbon monoxide gas has been described in a previous paper<sup>(1)</sup> In this paper certain modifications in the method of calibration are given

*Determination of the constants of the double wedge shaped trough* The troughs are made of patent plate glass cemented together by an acid resisting fusible glass, the whole being then mounted on a plate of glass,  $\frac{1}{4}$ -inch thick, by means of canada balsam A suitable sized trough has a length inside of 10 cm, a width inside of 2.2 cm, and a depth inside of 2.5 cm If the diagonal partition is made from plate glass 2 mm thick, then the thickness of the liquids held within the trough will be 2.0 cm One compartment of the trough should be marked " $O_2Hb$ " and the other " $COHb$ "

By examining in turn the reflections which occur at the six surfaces of the two long glass sides and the middle partition of the trough, it may be ascertained whether these surfaces are optically flat If they are found to be flat a steel mm scale may be used for measuring the thicknesses of the two wedges of fluid which will be contained in the trough when it is filled If they are not flat, the thicknesses of the two wedges of fluid must be ascertained by means of a micrometer microscope A suitable objective for this purpose is found to be  $1\frac{1}{2}$ -inch by Baker The eyepiece should magnify 10-15 diameters and should be fitted with a cross wire The constants of the trough are now determined as follows —On the inside surfaces of the trough some lycopodium particles are scattered, and the trough is placed on its side on the stage of the micrometer microscope so that the distance between the different glass surfaces can be determined The lycopodium particles on each of the glass surfaces are now focussed in turn, choosing those nearest the cross wire of the eyepiece This process is repeated for different positions of the trough, say at places 1 cm apart from one end of the trough to the other The trough should not be moved till the four measurements

*A, B, C and D* are complete for each position. *A, B, C and D* refer to the surfaces as shown in Fig. 1.



The results are tabulated in this manner:

Position of trough measured on scale fitted to microscope stage	Microscope readings			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
—	—	—	—	—
—	—	—	—	—
—	—	—	—	—
		etc.		

When the results are completely tabulated, three columns are calculated from them viz.:

Position of trough	<i>C-B</i>	<i>D-A</i>	$\frac{D-C}{D-C+B-A}$
—	—	—	—
—	—	—	—
—	—	—	—
			etc.

(*C-B* and *D-A* should both be nearly constant, and this permits of the results being checked.)

The last column is headed  $\frac{D-C}{D-C+B-A}$ ; this equals the p.c. saturation of the hæmoglobin with CO gas.

*The filling of the double wedge trough and the calibration of the spectro-scope.* The spectroscope is screwed to a board measuring roughly 15 cm. by 25 cm., as shown in Figs. 2, 3 and 4. In front of it are screwed guides for the double wedge trough and a mm. scale for determining its position. A very suitable source of illumination for the instrument is one supplied by Ogilvie for microscopic illumination. It consists of an Ediswan "Fullalyte" lamp mounted inside a metal box in the side of which is a small aperture. The board above referred to and the metal box containing the lamp are screwed to a bench (a place being selected that can be rendered fairly dark), their relative positions being such that good illumination of the spectroscope slit is obtained. Cardboard or metal screens are placed round the eyepiece of the instrument in such a way that bright light cannot enter the eye of the observer.

Into a litre flask is placed 20 c.c. of filtered ox blood and 380 c.c. of distilled water to which a trace of KOH solution has been added. Some of this solution is put into one of the compartments of the double wedge trough, the other compartment being filled with water. The double wedge trough is placed in front of the spectroscope and the spectrum of different thicknesses of the solution examined. The trough is adjusted until the  $\alpha$  band appears sharpest, which is usually found to be the case when the width of the  $\alpha$  band is equal to the bright area between the  $\alpha$  and  $\beta$  bands. See Fig. 2. The position of the trough is now observed and reference made to the previous table for values of  $A$ ,  $B$ ,  $C$  and  $D$ . From these  $\frac{(C-D)}{B-A} \times 100$  is calculated. This quantity is the amount of water in c.c. that has to be added to every 100 c.c. of solution of blood in order to bring it to the correct strength. With the trough in the optimum position 20 or more readings are taken of the wave lengths of the bands with the reversion spectroscope. The mean of these readings gives the micrometer readings for  $O_2Hb$ . The temperatures also should be noted, because it has been found that the wave length of these bands changes with temperature(3). Into each of two 200 c.c. flasks roughly 100 c.c. of the diluted blood solution are placed. Both are thoroughly shaken. One is labelled  $O_2Hb$  and is corked and placed on one side, the other is filled with carbon monoxide gas. The filling of the vessel with nearly pure gas is readily carried out by allowing it to bubble slowly under the layer of foam produced by the previous shaking. This vessel is now thoroughly shaken so as to saturate the blood solution. To the saturated



solution is added a few drops of ammonium sulphide solution; this absorbs the oxygen present and it therefore eliminates the risk of the carboxy-hæmoglobin being subsequently dissociated by accidental exposure to a bright light. Coal gas, if rich in carbon monoxide gas, may be used for saturating the blood solution, but it is better in this case to renew the gas in the flask three times and to shake very thoroughly after each renewal.

Into the compartment of the double wedge trough labelled "COHb" is poured some of the solution, prepared as described above, until it is about two-thirds full. The top edges are smeared with a little vaseline and a plane glass cover plate pressed into hermetic contact. When the joint is seen to be thoroughly gas tight the other compartment is filled with the oxyhæmoglobin solution contained in the second flask, and the trough is then placed in front of the spectroscope.

Different thicknesses of the two solutions are now set opposite the spectroscope slit so that the light entering the instrument has encountered different numbers of molecules of the two pigments. For each position of the trough the spectroscope is used for measuring the positions of the absorption bands until approximately 100 readings have been taken altogether. The readings should be tabulated thus:

(1)	(2)	(3)
Position of trough as read from scale	$\frac{D-C}{D-C+B-A}$	Spectroscope reading
—	—	—
—	—	—

The calibration curve is obtained by plotting the values in column (2) against those in (3).

*The accuracy of the determinations.* Experience shows that the mean values of ten readings taken by the same observer of the same blood sample seldom differ from one another by more than 1 Angstrom unit in wave length. Under favourable conditions a practised observer will obtain values the means of which differ by less than  $\frac{1}{2}$  A.U.

Reference to the typical calibration curve given in my earlier paper (1: Fig. 9) will show that  $\frac{1}{2}$  A.U. corresponds to rather less than 1 p.c. in the percentage saturation of blood with carbon monoxide gas. Duplicate sets of readings obtained by two observers using different instruments were seldom found to differ by more than 1 p.c.

The various physiological factors which affect the accuracy have been previously considered(2). Briefly it is found that an unfatigued observer working with a nearly dark adapted eye obtains the most

reliable results. Eyestrain and difficulty in focussing the spectra owing to insufficient power of accommodation, or to abnormal refraction, are both found to prejudice accuracy. It is of some physiological interest to observe that the accuracy with which the eye determines the wave length of the  $\alpha$  absorption bands of  $O_2$  and CO hæmoglobin is nearly as great as that with which it can observe when two sharply defined lines are set into coincidence. The cause of this considerable accuracy and the factors affecting it are to be described fully elsewhere.

When the calibration of the spectroscope is completed, and the instrument is being used for experimental purposes, readings should be made on the  $\alpha$  band of oxyhæmoglobin taken wherever possible from the same source and made at the same temperature as that of which the CO saturation is being determined. By this means errors can be eliminated which are due to temperature, to change in the eye of the observer, to change in the wave length readings of the instrument and to abnormality in the positions of the  $\alpha$  band of the blood sample.

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# VISUAL ACUITY AND THE RESOLVING POWER OF THE EYE. BY H. HARTRIDGE.

*(From the Physiological Laboratory, Cambridge.)*

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In the case of almost all optical instruments the value of the resolving power and the effects of aberrations in modifying that power are accurately known, because it is possible either by magnifying the image highly, or by some other method of analysis, to check theory, and to ascertain quantitatively what the intensity of illumination from point to point of the image may be. In the case of the eye the resolving power and effects of aberrations are not accurately known, because it has not hitherto been found possible to examine the image formed on the fovea in any greater detail than that provided by the foveal cones themselves, and this is quite insufficient for such a purpose. For this reason the subject of visual acuity is devoid of a firm basis. It is known that certain experimental values are obtained, but it is not known exactly why they should be obtained, rather than other values. One assumption has been made in the past, namely that the finite diameter of the foveal cones sets a maximal limit to the resolving power of the eye, and various calculations of the performance of the eye under different circumstances have been made on this basis. I shall show in this paper that this assumption has been made on insufficient grounds, and that the experimental performance of the eye does in certain cases exceed the calculated values thus made. In a previous paper<sup>(3)</sup> I showed that another method of attacking the problem existed, namely to calculate mathematically what the combined effects of diffraction and chromatic aberration on the images formed on the retina would be, in this paper I have extended the method to the calculation of the intensity of illumination of different foveal cones in the case of various test objects. By this means the visual acuity of the eye has been placed on a mathematical basis.

1. *Histological and optical limits to resolving power. Visual acuity for contours, their positions and movements.*

The resolving power of the eye has been found to be different by different methods for testing it. It was found by Hooke that two stars have to be separated by approximately one min. of arc in order that they should be seen separated by the eye. The distance between the bright bars of a grating in order that resolution should occur have been found to be of the same order, in general slightly less, as shown in the following table:—

Observer	Value at retina in $\mu$	Angle in seconds of arc
Lister ... ..	4.6	64
Hirschmann ... ..	3.6	50
Bergmann ... ..	3.8	52
Helmholtz ... ..	4.6	64
Uthoff ... ..	4.0	56
Cobb ... ..	4.6	64
	Mean 4.2	58

The resolving power of an optical instrument advanced by Fraunhofer in the case of the telescope, and Abbe in the case of the microscope—assuming pupil 3 mm., and white light—gives the least angle separating two objects which the eye can resolve as is slightly less still, namely about 41 secs. of arc.

The theory which has been put forward and which is commonly still held by physiologists and ophthalmologists, to account for the limitation of the resolving power of the eye, is that it depends upon the size of the cones in the fovea (that the two images should fall on two different cones, having an unstimulated cone between them). The size of the foveal cones has been differently estimated by different observers, as follows:—

Observer	Value in $\mu$ for diameter of cone
Max Schultze ... ..	2.8
H. Müller ... ..	less than 3.0
Merkel ... ..	3.0
Wadsworth ... ..	2.5
Kühne ... ..	2.0-2.5
Kölliker ... ..	4.5-5.4
Koster ... ..	4.4
Grief ... ..	2.5
Dimmer ... ..	3.0-3.5
	Mean 3.2

If the focal length of the eye be assumed to be 15 mm. then  $3.2\mu$  corresponds to an angle of 44 secs. of arc. On the smallest estimate ( $2\mu$ )

the angle separating the centres of two bright objects would be not less than 28 secs. of arc, on the largest ( $5.4\mu$ ) it would be 74 secs. of arc.

But the resolving power for certain objects is much greater than that found for double stars or for gratings; it is greater than that estimated by considering the eye as an optical instrument, and greater than can be accounted for on the theory of its dependence on the size of the cones. Thus Volkmann appears to have been the first to point out in 1863 that under certain circumstances, the acuity of the eye can greatly exceed the value of 1 minute of arc. He determined the smallest difference between the breadth of two white contours (on a black ground) that can be perceived by the eye. The least perceptible difference in angle that they subtend at the eye he found to be as little as 10 secs. of arc. Wulfling in 1892 confirmed Volkmann's observations and obtained values of 10 secs. to 12 secs. of arc. Hering in 1899 showed that the edges of two rectangles did not appear in line with one another if there was a relative displacement of approximately 10 secs. of arc. He gave to this ability of identifying such small displacements the term "sense of position" and he inferred that this property of the eye was one altogether different from the ability to resolve gratings, double stars or similar objects, to which the term "visual acuity" had been applied.

I have recently confirmed Hering's observations by means of a test object consisting of a white circle some 5 cm. in diameter, having at one point on its circumference, 10 mm. in length, a small excrescence, 2 mm. in width. This was mounted on a sheet of black paper so that the disc could be rotated into different meridians about a pin passed through its centre. The excrescence was placed in different meridians by an assistant, and was observed at increasing distances until the point was reached at which the position of the excrescence was correctly observed 8 times out of 10. The distance was found to be 130 feet, and this gave a value of 11 secs. of arc approximately. That is, a value corresponding almost exactly with that of Hering and previous observers. It is clear then from these experiments that so far as observing the relative position of objects is concerned, the discriminating power of the eye is very much greater than the limit set by the diameter of the foveal cones. Other observations with the coincidence method confirm this view. For example, it has been found by Brian and Baker<sup>(1)</sup> that the accuracy with which two lines can be set into coincidence with one another varies between approximately 8 secs. of arc and 10 secs. of arc. I have found, moreover, that objects with blurred outlines such as absorption bands can, under certain circumstances, be set into align-

ment with an accuracy of approximately 11 secs. of arc, that is, with an accuracy not very inferior to that obtained for sharp lines by Brian and Baker. The following table summarises these and other experiments.

Test	Observer	Angle in seconds of arc	Displacement of retinal images in $\mu$
Black lines	Bryan and Baker	12	·87
White "	"	9·5	·69
Split line	"	8	·58
Bisection	"	8·5	·62
Black lines	Hartridge	8·5	·62
"	Stratton	7	·51
Absorption bands	Hartridge	11	·79
		Mean 9·3	·67

Experiments show moreover that the eye is able to perceive narrow black contours seen against a bright ground when their edges subtend at the eye an angle of 4 secs. of arc. I have found, for example, that a black wire on a bright ground (not self luminous) is still recognisable when the angle which its edges subtend at the retina is approximately 3·6 secs. of arc, and in the table below are given values obtained by other experimenters for somewhat similar test objects.

*Acuity of vision for black line on bright ground.*

Seconds of arc				
Aubert ... ..	...	...	...	6
Smith Kastner ...	...	...	...	3·5
Hartridge ... ..	...	...	...	3·6
Hartridge and Owen	...	...	...	3·1

Spider's web  
Bright brass wire\*  
Black brass wire\*

Mean 4·0 = 20  $\mu$  at retina

\* Both mounted in front of white paper at such a distance that no shadow was cast.

Recent observations on the acuity of stereoscopic vision by Breton(2) have shown, moreover, this same high degree of accuracy, when the eye is used as an instrument for perceiving displacements of images. The values given in the following table show that the error of observation may be as low as 4 secs. of arc.

Observer	Angle in seconds	Retinal acuity in $\mu$
Pulfrich ... ..	10	·7
Heine ... ..	6-13	·4-·9
Bowdon ... ..	5	·35
Crawley ... ..	3*	·20
Breton ... ..	4	·28
Mean 8·2		·59

\* Probably an approximate value only (13).

In the following pages I propose to show how these various results can be harmonised.

2. *Relation between the form and position senses, and visual acuity.*

The questions which come up for consideration are therefore these. Is there any essential difference between the form and position senses and visual acuity? How is this high accuracy in the position and form senses to be explained, and lastly, how is it to be reconciled with the diffraction theory of light which, apart altogether from the diameter of the foveal cones, sets a limit to the fineness of structures which the eye should be capable of resolving?

It is well known that the images formed on the retina do not in any way correspond to those defined by the rules of geometrical objects. Calculations based on the diffraction theory of light show that the image of a point source of light consists of a diffraction pattern of somewhat complicated structure, and of a size which varies inversely as the diameter of the pupil. The effects of chromatic difference of focus and chromatic difference of magnification, have already been considered in a previous paper(3). It is sufficient to point out here that the effect of this aberration makes the image pattern still larger. It is found to vary directly as the diameter of the pupil. If, then, we imagine the pupil of the eye to be initially 3 mm. and that the pupil be increased, the effects of diffraction will be decreased so that the image formed on the retina will be improved, but the effects of chromatic aberration will be made worse and *vice versa*. There is apparently, therefore, some degree of compensation between these two effects.

In support of this deduction it may be pointed out that both Lister(4) and Cobb(5) found an increase in the acuity of the eye as the pupil increases from 0 to 3 mm. Thereafter both find a nearly constant acuity for the eye, that is, that for pupils between the average limits (3 to 6 mm.) nearly perfect compensation occurs. The aberration disc formed on the retina for every point source of light is therefore approximately constant in diameter, and it is possible to calculate the intensity of illumination at different parts of the disc, and therefore also the intensity of light stimulating the different cones of the fovea, if certain assumptions are made. These assumptions are that the eye suffers from no other important aberration beside chromatic aberration, and that this is present to an amount that calculation shows necessary, and that the eye suffers from diffraction in the same way as do other optical instruments of the same aperture.

Taking for example, the case of a bright narrow line on a dark ground, and assuming the diameter of the pupil to be 3 mm. and that the diameter of the foveal cones is  $3.2\mu$ . If then the intensity of illumination of that row of foveal cones, which exactly corresponds with the centre of the image of the line, equals 100, then the intensity of illumination of the rows of cones which lie on either side of the central row will be found by calculation to be 31 p.e.; and the next row of cones will be found to be illuminated by approximately 9 p.e., and so on. And it is not until the 6th row of cones is reached that the intensity of illumination has fallen as low as 1 p.e. In other words, even in the case of the image of a linear source, a large number of retinal cone elements receive stimulation, and it is not possible to think of any one cone or even any one small group of cones as being alone stimulated. We may then ask ourselves what it is that actually happens when such an aberration disc shifts its position on the fovea by a small amount. It would seem that the only change which takes place is that certain cones receive a slightly more intense degree of illumination than they did previously, whereas, in the case of other cones, there is a corresponding decrease. The least amount of displacement which will be perceived will be that which just causes a perceptible change in the intensity of light falling on a particular cone or a small group of cones. For example, suppose the image of a bright line to move approximately 44 secs. of arc. Then the row of cones which was previously receiving an intensity of 100 will now only receive an intensity of 31, whereas one of the rows of cones which previously received but 31 will now be receiving 100. Now we know that when large areas of the retina are being stimulated, the difference of intensity of 1 p.e. is perceptible under favourable circumstances, but it will be shown in the last section that any one cone or small group of cones is not able to respond to a difference of much less than 10 p.e. Applying this to the case which we have been considering above, we should imagine that since a change in angle of 44 secs. of arc results in a change in the illumination of two retinal cones of 69 p.e. that a 7-second of arc movement should be the least amount that the eye would be capable of perceiving, and this value fits approximately in with the results of coincidence and stereoscopic experiments referred to in the tables on p. 55.

On the theory given above of the unequal illumination of the cone, the fact that the eye can perceive the presence of a black object, the edges of which subtend an angle of about 3.5 secs. of arc, can be explained and the fact can be brought into harmony also with the conclusion based



on the diffraction theory that the black interval between two bright objects must not be less than 41 secs. of arc in order that they should be separately resolved. The clue to the explanation is to be found in a paper by Rayleigh(6). He points out, when there is a definite relation between the phases of two light sources, that a black line separating them should still be resolved even if it is narrower than that necessary under ordinary circumstances where no phase relation exists. Now in the case of a small black object lying on a white background (which is lit by scattering of light from some external light source, *e.g.* daylight) it would seem that there must be the necessary phase relation for resolution to occur, and that therefore Rayleigh's calculations explain the preception by the eye of black objects subtending these small angles.

Rayleigh gave the following formula for calculating the least width of a just visible black line when viewed against a self luminous ground

$$\frac{I_1 - I_0}{I_0} = \frac{2a}{\pi}.$$

This may be put "perceptible percentage decrease of illumination multiplied by angle subtended at the eye by the lines of a grating for resolution = angle subtended at the eye by edges of just visible black line." He showed that for a grating and a line placed in front of a sodium flame and viewed by the eye through a pin hole, the angle ratio was 9.1 to 1, and therefore by substitution in the above formula the percentage decrease in illumination is equal to 11 p.c. This value of 11 p.c. fits in with that required to explain a position sense of approximately 7 secs. of arc as shown above.

Taking similarly the case of objects viewed in front of backgrounds illuminated by borrowed light (*e.g.* white paper illuminated by daylight) Rayleigh gave the formula

$$\frac{I_1 - I_0}{I_0} = \frac{4a}{\pi}.$$

This may be put "perceptible percentage decrease in illumination multiplied by angle subtended at the eye by the lines of a grating for resolution, = four times angle subtended at eye by edges of just visible black line."

As stated above Rayleigh's experiments show that the least perceptible decrease in illumination is approximately 11 p.c. The angle for resolution of a grating is about 58 secs. of arc. The angle of a just visible black line should therefore be about 1.54 secs. of arc with monochromatic light. The lowest value which has been obtained in daylight by Owen and myself is 3.1 secs. of arc. Daylight would not be expected

to give such good values as monochromatic light because of the chromatic aberration of the eye. Thus Luckiesh<sup>(7)</sup> has shown improvements varying between 1 p.e. and 300 p.e. in the visual acuity when monochromatic light was substituted for ordinary light. Other aberrations of the eye would also cause the experimental value to fall somewhat short of the theoretical one. We conclude, therefore, that the observed performance of the eye fits in closely with the performance to be expected according to the diffraction theory of light.

In the case of bright objects on black grounds, many writers have pointed out that the limit met with in the case of black objects is not present; the controlling factor being not the width of the bright object but the intensity of the light which it sends into the eye, cf. Parsons<sup>(12)</sup>. Thus ultramicroscopic structures are rendered visible if their illumination is sufficiently intense as Sidentopf has shown. Rayleigh pointed out that the observation that the eye is capable of perceiving a single black or white line when it subtends an angle at the eye as little as say 3 secs. of arc, does not alter the value of the limit set to the closeness of two objects or the bars of a grating for them to be resolved. That is, that the older visual acuity limits still hold.

Now it seems to me that the importance of the resolution of double stars to the astronomer, and grating-like structures to the microscopist, has given to these types of test object a quite undeserved importance. In ordinary visual observation such types of object are seldom met with, and even such geometric designs as the letters of the alphabet are but distantly related to such test objects. On the other hand, contours of all kinds are met with to a very great extent in ordinary visual observation, and it would appear that the important phenomenon of simultaneous contrast has been specially developed in order that the appreciation of the existence of contours should be facilitated. The narrowness of a contour, of which the eye can perceive the existence, is therefore a matter of far greater importance in vision than the fineness of a grating or the closeness of two double stars that can just be resolved.

### 3. *The difference threshold of the foveal cones for light intensity.*

It is surprising to find how little is known concerning the values for the difference threshold on which the appreciation of the form of external objects depends. In this section the probable values for the difference threshold will be deduced for the various types of test object referred to above, namely, a single black line, a grating, a double star, coincidence of two black lines, the coincidence of two bright lines, stereoscopic

vision, sense of position and lastly the coincidence of two absorption bands.

*Single black line.* This is the case examined by Rayleigh of which mention has already been made in the previous section. He estimated that a just visible black line produces a decrease in intensity of light on the retina of 11 p.c. Now this value is that corresponding to the centre of the geometrical image and is not the average value corresponding to a foveal cone. To obtain this it is necessary to calculate the intensity values from point to point of the geometrical image, and then to average these values for each cone. Such point to point values are not obtainable from Rayleigh's formula, neither is any account taken of the effects of chromatic aberration which the eye is known to suffer from.

The following table gives the result of calculating the combined effects of chromatic aberration and diffraction for a black line, the retinal image of which is  $\cdot 76\mu$  wide, seen against a self luminous ground.

	Distance from centre of image in $\mu$	Relative % intensity of light		Average intensity on cone*
	+4.56	98.4	}	... 96 %
	+3.80	97.8		
	+3.04	96.9		
	+2.28	94.7		
	+1.52	90.1		
	+ .76	84.1	}	... 83 %
centre	$\pm 0$	81.2		
	- .76	84.1		
	-1.52	90.1		
	-2.28	94.7		
	-3.04	96.9	}	... 96 %
	-3.80	97.8		
	-4.56	98.4		

\* Assuming cones to have a diameter of  $3.2\mu$ .

The table shows that if 100 is the value of the uninterrupted illumination there should be an intensity of 83 p.c. on the row of cones corresponding to the centre of the image, and an intensity of 96 p.c. in the row of cones on either side of the central row, *i.e.* a difference between the rows of 13 p.c. Now experiment shows that a line subtending 4 secs. of arc approximately is seen against a ground illuminated by borrowed light. But to be seen against a self luminous ground a line doubly as wide would have to be employed, according to Rayleigh's formulae, *i.e.* one subtending 8 secs. of arc approximately. Such a line would form a geometrical retinal image of  $\cdot 585\mu$  width and this would produce a correspondingly less marked intensity difference on the cones. Calcula-

tion shows that the difference threshold of stimulation between the two rows of cones would have to be 10 p.c. approximately, for the perception of such a black line to occur. The agreement between this value and Rayleigh's (11 p.c.) should be noted.

*Grating.* The experimental value of the acuity of the eye for this test object (cf. p. 53) is approximately 58 secs. of arc. Now resolution of such a structure can occur when there is a perceptible difference in the stimulation of neighbouring retinal cones. This is found to be the case when one cone on which a bright bar falls, has on either side of it two cones on which images partly consisting of dark bars fall. For example this occurs with the experimental values quoted above because while the cone corresponds to 44 secs., the neighbouring dark bars of the grating correspond to 58 secs., that is, that there is some 7 secs. overlap of the dark bars on either side of the cone on which a bright bar exactly falls.

Calculating the intensity at different points of the image, chromatic aberration and diffraction being both taken into account, the following values were obtained.

Distance from centre of image on retina in $\mu$		Relative intensity	Average intensity on cones*
$\pm .00$	(centre of bright bar)	57.0	... 100 %
+ .38	" " " "	55.5	
+ .76	" " " "	52.5	
+ 1.14	" " " "	46.0	
+ 1.52	" " " "	44.5	
+ 1.90	" " " "	41.2	... 80 %
+ 2.28	(centre of black bar)	41.2	
+ 2.66	" " " "	44.5	
+ 3.04	" " " "	46.0	
+ 3.42	" " " "	52.5	
+ 3.80	" " " "	55.5	... 100 %
+ 4.18	(centre of bright bar)	57.0	
4.56	etc.	55.5	

\* Assuming foveal cones to have a diameter of  $3.2 \mu$ .

A difference of intensity of illumination of 20 p.c. would therefore be expected between the cones corresponding to the exact centres of the bright and dark bars. It would only happen by chance that neighbouring cones would correspond exactly to the centres so that a somewhat lower difference of intensity of illumination would probably be found in practice. It is interesting to note that according to Rayleigh's values a self luminous grating is more easily resolved by the eye than one illuminated by borrowed light. The converse is the case for the resolution of a single narrow black contour.

## A. Cases in which there is a constant difference of intensity.

Single black line on white ground	13 %
Grating ... ..	20 %
Double line... ..	12 %

## B. Cases in which there is a variation of intensity.

Coincidence ... ..	22 %
Position ... ..	32 %
Stereoscopic vision ... ..	12 %

between the intensity at one moment and that at another. These values are otherwise fairly concordant, allowing (a) for experimental error, (b) for the fact that the observers who have measured the resolving power of the eye for the various test objects have been different and (c) for the difficulty met with in making even approximate calculations of the intensity in different parts of the retinal image.

4. *Factors affecting the difference threshold for light intensity.*

In the calculations I have given of the intensity of illumination at different parts of the retinal image, the effects of chromatic aberration and diffraction have alone been considered. But it is well known that even the eye of the emmetrope suffers in addition from other aberrations, *e.g.* spherical aberration, astigmatism, disobedience to sign condition, etc. The latter may, however, be excluded since it should not appreciably affect the definition of images formed near the optic axis. The other two aberrations should also have but a small effect so long as the pupil diameter does not greatly exceed 3 mm. It remains however to consider four further factors (a) the effect of light entering the eyeball other than through the pupil, (b) the effect of light entering the pupil and suffering multiple reflections at the different surfaces bounding the optical media, (c) the effect of light entering the pupil and suffering irregular refraction owing to slight irregularity in the optical surfaces, (d) the effect of light reflected from the black parts of the test objects.

With regard to (a), it is well known that some light enters the eyeball, *e.g.* through the sclera and choroid, and thus illuminates the retina by scattered rays. It is improbable, however, that this light would be considerable in amount unless strong direct light was falling from one side on to the eyeball, and it can be fairly assumed that an experimenter aiming at the best results would avoid such circumstances.

With regard to (b), it can be said that taking the optical surfaces in turn, the air-cornea surface (R.I. 1-1.34) will reflect light in an outward direction only; the cornea-aqueous surface (R.I. 1.34-1.334) will reflect practically no light because the difference in refractive index is so small;

the aqueous-lens surface (R.I. 1.334-1.37) reflects but a small amount of light because of the low refractive index of the peripheral layers of the lens; it will be remembered further that the succeeding layers increase in refractive index gradually till the nucleus is reached with a R.I. of 1.41, and that after that is passed the layers are of gradually decreasing R.I. till the periphery is again reached. This peculiar structure gives to the whole lens an equivalent R.I. of 1.42 in a manner described in a previous paper<sup>(1)</sup> and yet the light scattered in passing through the lens must be exceedingly small because at no part of its course does it encounter a big change of refractive index. There are grounds for believing, therefore, that the amount of light suffering multiple reflection at the eye media must be very small.

With regard to (c) it is well known that images of objects reflected in the surface of the normal cornea show small irregularities. These irregularities, unless they are exactly corrected by the subsequent surfaces through which the rays pass, will cause some scattering and will prevent the exact union of the rays forming the image of a point source. It is not difficult to observe with the normal eye the effects of irregularities such as these if a bright point source of light be examined in a dark room, for a halo consisting of a large number of spectra will be seen surrounding the source.

With regard to (d), it is well known that the majority of black objects reflect about 5 p.c. of the light that falls on them and therefore some such value presumably applies also to the black components of the objects used for testing the visual acuity. This light will tend to diminish the difference in illumination at different parts of the retinal image.

The effects (a), (b), (c) and (d), though the effect of each alone is probably small, combine to make the values given in the table, p. 64, too high. This table is moreover for observations in which a small group of cones only is affected. When a large group of cones is affected the values are much less. Thus for example with the  $\alpha$  band of oxyhæmoglobin spectrophotometric measurement shows that at the steepest part of the absorption gradient a 1 p.c. change in intensity occurs for a 2 A.U. change of wave length. Since these bands can be measured with an accuracy of 1 A.U. a  $\frac{1}{2}$  p.c. intensity change must be sufficient for a noticeable difference to be produced. Now according to Weher's experiments a 1 p.c. difference in the intensity of illumination of two neighbouring bright areas was just noticeable. Helmholtz found however that the value is more nearly 0.6 p.c. and this agrees well with the values I have just given for absorption band measurements.

Of the factors which affect the value of the least perceptible difference of light intensity both for small and large groups of cones, intensity itself is probably the most important, for it is well known that under diminished illumination visual acuity for all types of test object is reduced and a greater difference in illumination is necessary for that difference to be observed. It is also probable, if the intensity of illumination is increased beyond a certain amount, that the optimum would be passed and a decrease in acuity result.

It may be noticed that diminished illumination of a test object only involves a change in the absolute intensity of the different parts of the image, and does not involve any relative change. Since then a test object which is visible at one intensity ceases to be visible when the intensity is reduced, it follows that a percentage difference of intensity recognised at one intensity is not recognised at a lower one. This is another example of disobedience by the eye of Weber's law.

A further factor which might be expected to change the value of the difference threshold is the degree of adaptation of the eye. It has been shown that visual acuity increases in the light adapted eye. The observations for small retinal areas have been summarised by Parsons<sup>(9)</sup>. Cobb<sup>(10)</sup> has found a similar change both for small and large retinal areas. So far, however, the results obtained by experiment have only been qualitative, thus there are no values from which a curve can be plotted showing graphically, how the difference threshold changes with changes in retinal adaptation.

#### SUMMARY.

1. It is known that the resolving power of the human eye varies with different objects. Thus for double stars the limit is about 60 secs. of arc, and for the narrowest visible black contour it is about 4 secs. of arc. The latter is much less than that calculated in optical ground, viz. 41 secs. of arc. It is also known that in practically every case in which the resolving power of the eye greatly exceeds 60 secs. of arc, the visibility of a contour is involved.

2. The way in which the optical limit to the resolving power is exceeded in the case of contours is explained on the basis of a paper by Rayleigh.

3. The way in which the histological limit to the resolving power is exceeded in the case of contours is considered in detail. The basis of this is the calculation of the intensity of illumination from point to point of the images of different contours. From these values the intensity of

illumination of the cones of the fovea which are affected by the image can be approximately ascertained. In this manner the difference of intensity of illumination of neighbouring cones when a stationary image is falling, or the change of illumination which follows the movement of an image is obtained. Since the experimental values for the different images are known the probable smallest perceptible percentage difference of illumination can be calculated.

The results (cf p 64) show that where there is a constant difference of intensity there is usually a lower value for the difference threshold than when there is a variation of intensity. This would be expected since a memory process is required in the second case which is not required in the first.

Also as is shown in the text the latter is more liable to be vitiated by irregular eye movements.

4 Values are given for the least perceptible percentage difference of illumination of large groups of cones

Coincidence of two absorption bands	0.5 p e
Comparison of two illuminated areas	0.6 p e

These values are altogether different from those obtained in the case of small groups of cones. The difference will be reduced slightly if the effect of scattered light within the eyeball is taken into account, this will arise from light that has penetrated the sclera, light irregularly refracted by the optical surfaces, *e.g.* by the cornea, and light that has suffered multiple reflection within the eye. The effects of these factors taken together cannot be large so that a considerable amount of the above difference remains to be explained.

Other factors shown by experiment to affect the difference threshold are intensity and retinal adaptation.

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# THE RELATION BETWEEN THE EXTERNAL WORK PRODUCED AND THE TIME OCCUPIED IN A SINGLE MUSCULAR CONTRACTION IN MAN.

By HARTLEY LUPTON<sup>1</sup>.

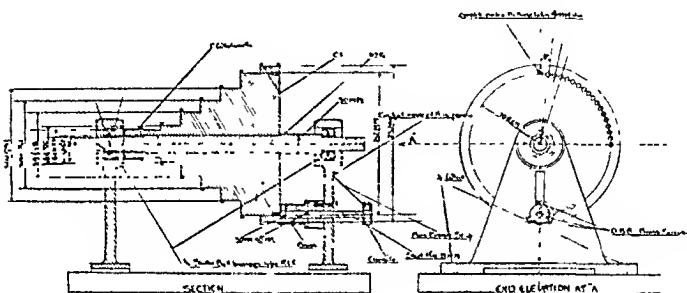
*(From the Physiological Laboratory, Manchester.)*

In a recent paper it was shown experimentally by A. V. Hill<sup>(1)</sup> that the maximum work done in a single muscular contraction in man depends in a simple and exact manner upon the mass moved or upon the time occupied in the movement. The method employed was to oppose the force exerted by the muscle to the inertial reaction of a flywheel pulled by a cord wound round one or other of a series of pulleys of different size, the kinetic energy developed in the wheel being measured by a standard tachometer. One possible source of error in these experiments, which was shown however<sup>(1, p. 41)</sup> to be of comparatively small importance, was that the flywheel was released at the moment the contraction commenced, and was allowed therefore to travel a short distance before the force exerted had reached its full extent. A more important objection to the method was that the time occupied in the contraction was not observed directly, but calculated on assumptions which were admittedly approximate. This paper records a re-investigation of the phenomena described, with a technique to which these objections do not apply.

In order to ensure that the movement did not commence before the maximum force of the muscle had been developed, a "quick release" mechanism was designed and made by Mr A. C. Downing, of the Department of Physiology, on the following plan (see Figs. 1 and 2). A steel pin passed through one of the main bearing supports of the wheel and entered one of many small holes 4 mm. in diameter drilled in the side of the wheel along a circle of radius 10.5 cm., whose centre was the axis of the wheel. This pin was released only after the tension had been fully developed, thus allowing no shortening to occur before the development of the maximum force of the contraction. Such a "catch" mechanism for allowing the development of the full muscular effort before release of the limb or body, and the effect upon the efficiency of the contraction,

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have been described in the case of certain jumping insects by Haycraft (2). For recording directly the time occupied in the contraction another



Figs. 1 and 2.

mechanism was used, also designed and made by Mr Downing. A pointer, actuated by an electromagnet, wrote upon a revolving drum driven at a constant speed, and marked by a time-marker in  $\frac{1}{8}$  secs. The electromagnet was first short-circuited through a phosphor-bronze spring attached to the pin, contact being broken instantly and the current allowed to run through the magnet, on removing the pin. The moment of release therefore was marked by the downward movement of the pointer. The pointer remained at the lower level until the circuit was again short-circuited, at the end of the contraction, by the hand of the subject knocking down a lever which caused electrical contact between two insulated points connected across the terminals of the electromagnet. After the preliminary "Ready," the word "Go" was given, and then after a sufficient short interval the pin was sharply withdrawn, the work being measured as usual with the tachometer, and the duration of the movement being read off afterwards from the record on the drum.

Readings of the work performed and of the time taken were made successively on each of the eight pulleys. In about half the experiments the starting-point was the pulley with the largest equivalent mass, readings being taken on each pulley until the one with the smallest equivalent mass was reached. The readings were then repeated in the reverse order. In the other experiments the starting-point was the smallest equivalent mass (largest diameter pulley). By these means errors due to fatigue were entirely eliminated. Instead of cord was

used a length of thin Bowden-brake wire with a loop at each end. Through one loop passed a brass rod to be used as a handle; the other loop slipped over the small peg in the circumference of the pulleys. The length of the wire was so arranged that a few centimetres still remained on the circumference of the pulley at the end of the contraction. This dropped off as the wheel continued its revolutions, and thus it was made certain that the full amount of work was obtained from the contracting muscles. The use of this wire necessitated a re-calibration of the instrument. The diameter of the wire was only 1 mm.—much smaller than that of the cord previously used—and as the effective radius of a pulley is altered by any variation in the diameter of the cord, such change will affect the equivalent masses of the pulleys, particularly those of the smaller diameter pulleys, where the diameter of the cord becomes an appreciable fraction of the whole. The calibration of the instrument was carried out in the usual manner. Table I gives the radii and equivalent masses of the pulleys.

TABLE I.

Pulley	1	2	3	4	5	6	7	8
Effective radius, cms.	1.535	2.148	2.779	4.76	6.59	8.41	10.69	11.72
Equivalent mass, kilos	681	348	208	70.8	36.94	22.69	14.04	11.67

*Results.* Out of a large number of subjects whose records of work produced on the different pulleys were taken, the most consistent subject was chosen and in nine experiments the time of contraction on each pulley was recorded along with the external work produced. Table II gives the values of the work obtained on each pulley in these nine experiments, together with the values obtained in one other experiment on the same subject in which the times were not recorded.

TABLE II.

MASS: kilos	681	348	208	70.8	36.94	22.69	14.04	11.67
WORK: kgr.-metres	9.70	9.17	8.63	7.46	6.50	5.63	4.86	4.58
Mean value (10 observations)								
Probable error of mean	±0.08	0.06	0.07	0.04	0.05	0.04	0.03	0.03

The probable error of the mean value was calculated as follows. If  $d_1, d_2, \dots, d_n$  were the deviations of the 10 single observations from their mean value, the probable error of the mean was

$$\pm 0.67 \sqrt{\frac{\bar{d}_1^2 + \bar{d}_2^2 + \dots + \bar{d}_n^2}{n(n-1)}}$$

Fig. 3, shows the mean values of the work plotted against the equivalent mass of the pulley.

It was shown by A. V. Hill<sup>(1)</sup> that the relation between the maximum

work done and the duration of the movement is given by an equation  $W_0 - W = k'/t$ : in a later paper(3) he adopted the similar but more

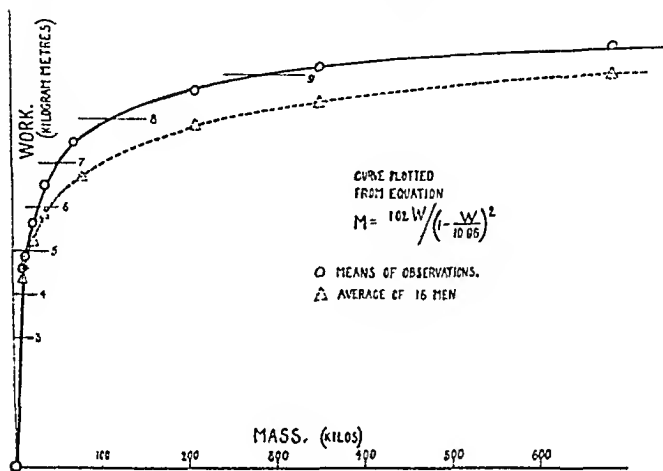


Fig. 3.

convenient formula  $\frac{W_0 - W}{W_0} = \frac{k}{t}$ , which implies that the fraction of the theoretical maximum work wasted in overcoming the frictional resistance of the muscles and limb is proportional to the speed with which the process is carried out. It was shown also that the relation between the maximum work and the equivalent mass can be expressed by the formula

$$M = \frac{k^2}{2l^2} \cdot W / (W_0 - W)^2,$$

which in the second and revised notation becomes

$$M = \frac{k^2}{2l^2} \cdot W \left( \frac{W_0 - W}{W_0} \right)^2,$$

$l$  being the length of the pull. Putting  $\frac{k^2}{2l^2} = K$  this becomes

$$M = K W / \left( 1 - \frac{W}{W_0} \right)^2.$$

In Fig. 3 is plotted a theoretical curve with  $W_0 = 10.96$  kgr.-metres, and  $K = 1.02$ : it is seen that the agreement is excellent. In addition to this curve for the most consistent subject, the average values of the work obtained on each pulley from sixteen men chosen at random are

given in Table III and with these values an average curve is plotted in Fig. 3 (dotted line).

TABLE III.

Mass	681	348	208	70.8	36.94	22.69	14.04	11.67
Work	9.08	8.38	7.84	6.67	5.82	5.20	4.66	4.37

Considering now the relation between the times occupied in the contractions on the different pulleys and the external work produced, the results obtained in nine complete experiments on the most consistent subject are given in Table IV.

TABLE IV.

WORK: kgr.-metres.	9.70	9.17	8.63	7.46	6.50	5.63	4.86	4.58
Mean value								
TIME: secs. Mean value	2.16	1.60	1.25	0.83	0.66	0.55	0.51	0.46
(9 observations)								
Probable error of mean	$\pm .013$	$\cdot 007$	$\cdot 013$	$\cdot 004$	$\cdot 007$	$\cdot 005$	$\cdot 007$	$\cdot 003$

The mean values of the times are plotted against the mean values of the work in Fig. 4. In this figure is shown also a theoretical curve

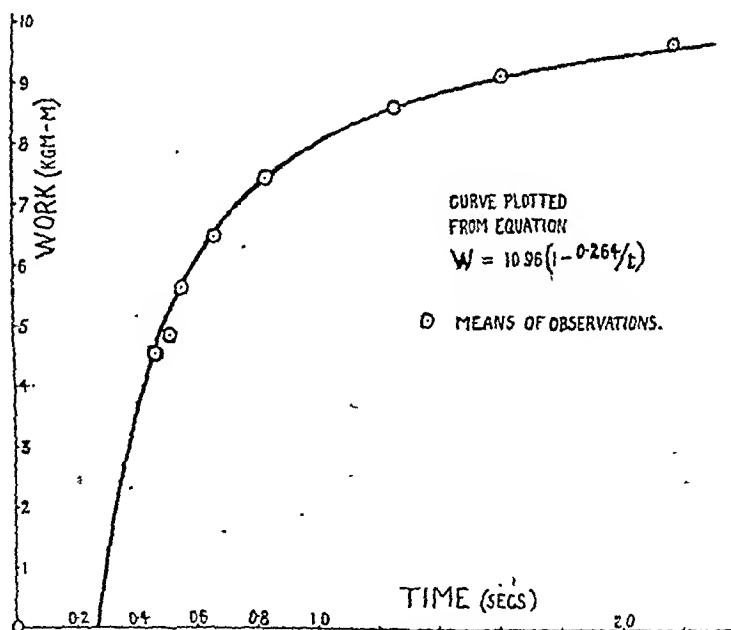


Fig. 4.

obtained from the equation  $W = W_0(1 - k/t)$ , with the values  $W_0 = 10.96$  and  $k = 0.264$ . The agreement again is excellent.

Assuming that the force maintained throughout the contraction is

constant, an assumption shown experimentally by A. V. Hill to be approximately correct, the constants  $K$  and  $k$ , determined experimentally as above, should be connected by the relation  $K = k^2/2l^2$ . In determining  $K$  experimentally we have employed kilograms and kilogram-metres as units so that in absolute c.g.s. units  $K$  becomes  $1.02/98100$ . Hence we have the equation  $1.02/98100 = (0.264)^2/2l^2$ , from which we find  $l = 58.0$  cm. In the case of the subject investigated the observed mean length of the contraction was 61 cm. The agreement is fairly good: the divergence is due partly, no doubt, to the approximate nature of the assumption that the force maintained throughout the contraction is constant: it may be accounted for largely however by the fact that the lever which, by being knocked down, marked the end of the contraction, could not be placed exactly at the end of contraction. On the whole therefore we may say that the theory quite adequately covers all the observed facts. It will be observed that the same value of  $W_0$  was used for both equations.

A similar set of experiments was performed on the "standard subject" of the original determinations of A. V. Hill(1). Eight experiments were performed in which the time of contraction was recorded along with the external work done. The mean values obtained are given in Table V.

TABLE V.

Equivalent mass, kilos	Work, kgr.-metres (mean)	Probable error of the mean	Time of contraction (secs.), mean	Probable error of the mean
681	9.31	$\pm 0.00$	2.09	$\pm 0.13$
348	8.76	$\pm 0.04$	1.54	$\pm 0.07$
208	8.24	$\pm 0.08$	1.25	$\pm 0.13$
70.8	7.20	$\pm 0.05$	0.79	$\pm 0.04$
36.94	0.19	$\pm 0.05$	0.62	$\pm 0.07$
22.69	5.50	$\pm 0.04$	0.52	$\pm 0.04$
14.04	4.96	$\pm 0.05$	0.45	$\pm 0.04$
11.67	4.64	$\pm 0.03$	0.42	$\pm 0.04$

These results are fitted with the same accuracy as in the case of the other subject by the equations (i)  $W = W_0(1 - k/t)$ , with  $W_0 = 10.54$  and  $k = 0.260$ ; and (ii)  $M = KW/(1 - W/W_0)^2$ , with  $W_0 = 10.54$  and  $K = 0.987$ . Here again the value of  $l$  calculated from the equation  $0.987/98100 = (0.260)^2/2l^2$ , viz.  $l = 58$  cm., is slightly less than the value actually observed, viz.  $l = 60$  cm., no doubt for the same reason. On this "standard subject" A. V. Hill's original observations gave a  $k$  of 0.242, and a  $W_0$  of 11.18. The difference in  $W_0$  is of no importance; it may be attributed to a small change in the bodily condition of the subject: the two values of  $k$  are very nearly the same, the small difference

being due probably to the fact that in A. V. Hill's paper the times were *calculated* for the full extent of the pull, while in the above experiments they were directly observed over a slightly smaller range of movement.

### DISCUSSION.

The results of these experiments are in agreement with the conclusions of Hartree and A. V. Hill(4) and of A. V. Hill(1, p. 40) that the external work done in a muscular contraction is diminished through viscosity by an amount depending upon the velocity of shortening. Rapid movement means large internal friction and large loss of energy as heat, the external work produced by the muscular contraction being thus considerably less than the original potential energy possessed by the muscle. An interesting problem, on which it is impossible at present to throw any light, is presented by the case of the rapidly moving muscles of insects. In the muscles of the human arm a movement completed in 1 second wastes 26 p.c. of its energy in overcoming the frictional resistance of the muscle itself. a movement completed in  $\frac{1}{2}$  second some 52 p.c., until, in a movement completed in about 0.26 sec., no external work can be done at all. Yet the movements of the wing muscles of insects are enormously more rapid than this. Presumably the minute structure or the anatomy of the muscle is modified in some way to allow these movements to occur without the extreme wastefulness associated with rapid movement in man.

In conclusion I wish to express my thanks to Prof. A. V. Hill for inspiration and helpful suggestions. My thanks are also due to Mr A. C. Downing for the drawing of Figs. 1 and 2 and to Mr W. Baker and Mr Downing for acting as subjects.

### SUMMARY.

Improvements are described in the method by which the maximum work of human muscles contracting against the inertial reaction of a flywheel and the time occupied in the movement can be measured. The measurements confirm the accuracy of the equation  $W = W_0 (1 - k/t)$ , where  $W$  is the work done in a maximal movement occurring in time  $t$ .  $W_0$  is a constant (the theoretical maximum work) and  $k$  another constant probably depending on the viscous resistance of the muscle to a change of form. It is shown also that the equation  $M = KW/(1 - W/W_0)^2$  relating the work  $W$  to the mass  $M$  opposing the contraction is very

xactly obeyed,  $K$  being another constant related to  $k$ . Various consequences of these relations are discussed.

The expenses of this research have been borne in part by a grant from the Royal Society to Prof. A. V. Hill.

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THE MEASUREMENT OF THE TENSION OF OXYGEN  
AND CARBON DIOXIDE IN THE BLOOD OF THE  
PULMONARY ARTERY IN MAN. BY ALFRED C. RED-  
FIELD, ARLIE V. BOCK AND J. C. MEAKINS.

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and the Harvard Medical School.)*

THE estimation of the volume of blood expelled from the heart in unit time by the principle introduced by Fick(1) requires a knowledge of the quantity of oxygen or carbon dioxide in the mixed venous blood which is pumped by the right heart into the pulmonary artery. The calculation of the diffusion constant describing the rate of passage of oxygen across the pulmonary epithelium under unit difference of pressure in accordance with the method of Bohr(2) involves data concerning the pressure of oxygen in the blood which is flowing into the lungs. In experiments upon animals such information can be obtained directly by standard methods of analysis from samples of blood obtained by puncture of the right ventricle. For experiments on the human subject resort must be had to a less direct method.

A number of ways have been devised purporting to give with some accuracy the tension either of the oxygen or of the carbon dioxide in the mixed venous blood entering the pulmonary capillaries. In using such data for estimating the per minute volume output of the heart use must be made of a curve relating the quantity of gas taken up by a given volume of blood when it is exposed to any partial pressure of the gas within the physiological range of variation. Since it has been shown by Bohr, Hasselbalch and Krogh(3) that the position of such a curve in the case of oxygen is profoundly modified by the quantity of carbon dioxide present, and by Christiansen, Douglas and Haldane(4) that the position of the curve relating tension and content of carbon dioxide is influenced in a similar way by the degree of oxidation of the blood, it is desirable that the tension of both gases in the mixed venous blood be estimated synchronously. In the methods heretofore in use, with the exception of that employed by Douglas and Haldane(5), this fact has either been overlooked, or some assumption has been made concerning that gas whose tension was not measured. In developing the present method this consideration has been determinant; in addition,

we have been desirous of finding a procedure which could be carried through in a short period of time and which introduced no manipulations which could not be carried out by trained subjects when exposed to conditions of severe anoxæmia.

The principle involved is simply that the partial pressure of any gas taken into the alveoli of the lungs will tend to come into equilibrium with the tension of that gas in the blood which is entering the lung from the pulmonary artery; and this will be true of each gas present. Practically, equilibrium cannot be approached with such blood as traverses the pulmonary artery under any standard condition unless the gas mixture is close to the equilibrium point to start with, for in the requisite time the blood which was exposed to the gas mixture at the beginning of the period will have completed its circuit of the system and will return to the lung in an abnormal condition. It is necessary, consequently, that the sample of gas should not be held in the alveoli for more than 30 seconds; actually a shorter period will suffice for our method. Since a true equilibrium cannot be obtained generally, it was hoped that by observing the course of the change in the partial pressure of oxygen and carbon dioxide as equilibrium was approached, that some estimate could be made of the final equilibrium point. Consequently, samples of gas were taken into the lung, held there for a few seconds, and then about half the lung contents were expelled and a sample of the last portion of gas leaving the lung was collected and analysed for oxygen and carbon dioxide. The remainder of the gas was then held in the lung for 5 or 10 secs. more and then expelled and a second sample collected for analysis. When data obtained in this way are plotted graphically as in Fig 1 in which the oxygen pressure is laid off on the abscissa and the carbon dioxide pressure on the ordinate, and straight lines are drawn through the pairs of points representing tensions of the gas before (*A, A' A''*) and after (*B, B', B''*) being held in the lungs for 5 or 10 secs., it is found that the lines meet at a point, or very nearly do so. One would expect them to do this on the assumption that these straight lines represent the actual course of the change in tension of the gas as it approaches equilibrium with the blood entering the lung from the pulmonary artery. The point of meeting should represent the tension of oxygen and carbon dioxide in that blood. It remains a question how far this assumption is justified. The linear course of the changing tensions of the gas in the alveoli, which is indicated by the figure, implies that at any moment the change in tension of both gases is, in the case of each, affected equally by the difference in tension between that gas at any moment and its

tension at the final condition of equilibrium; *i.e.* at the tension of that gas in the blood entering the lung from the pulmonary artery. Such a

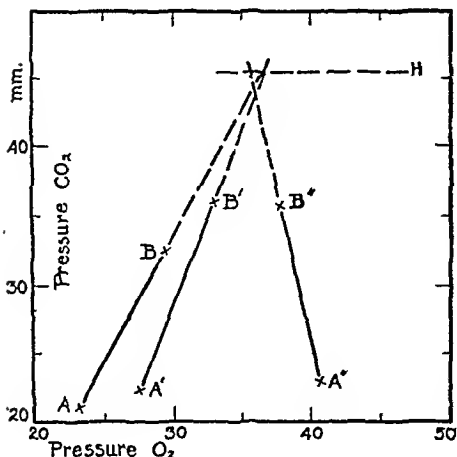


Fig. 1.

Fig. 1. A.C.R. The horizontal line, *H*, is the  $\text{CO}_2$  isopleth determined by the Henderson and Prince method.

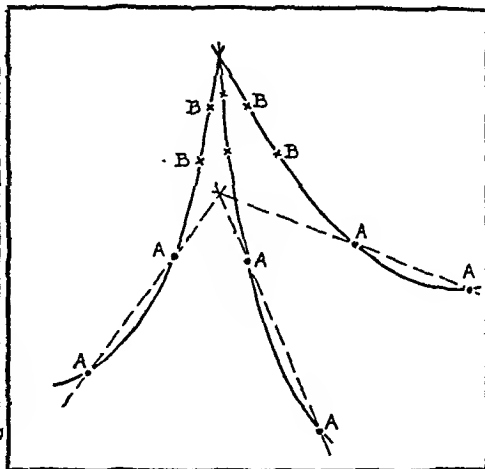


Fig. 2.

Fig. 2. See text.

condition seems *a priori* improbable for two reasons: (a) because the diffusion constant of carbon dioxide is generally considered to be many times as great as that of oxygen, and consequently, carbon dioxide should approach an equilibrium with many times the rapidity of oxygen, and (b) because while equilibrium is being approached the effective tension of gas in the blood is its mean tension during the time it is traversing the alveolar capillaries, which coincides with the tension in the mixed venous blood only at the time when equilibrium is established between the blood and the alveolar gas. For these reasons one would expect the course taken by the changing values of oxygen and carbon dioxide tensions to be curved lines when represented graphically, somewhat as in Fig. 2. It can be shown however that this is not the case.

If the course of changing tensions of  $\text{CO}_2$  and  $\text{O}_2$  in the alveolar gas is a line which is not straight or nearly so, then the straight line drawn through pairs of points on such a curve will not coincide except by chance, and should such straight lines drawn through pairs of points on several different curves chance to coincide—as in Fig. 2 *A*—the point of coincidence will occupy a position which will differ depending on whether the pairs of points are taken near to (Fig. 2 *B*) or remote from (*A*) the

true point of equilibrium. Consequently, it is only necessary to determine the point of transection of three lines drawn through pairs of points of which the initial member is obtained from samples of gas, the composition of which differs markedly from the expected equilibrium point, and then determine the point of transection of lines obtained from gas samples having an initial composition approaching closely to that indicated by the first point of transection. If the points of transection obtained in the two cases coincide, it may be concluded that the course of the change in composition of any sample is a straight line and the transection point represents the true tensions which the gases would have if equilibrium were established. Fig. 3 illustrates such an experiment and shows that

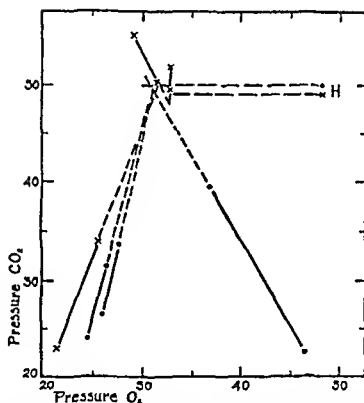


Fig. 3. A.V.B. Dots (•) indicate observations made July 7. Crosses (x) observations made July 8. *H* indicates the CO<sub>2</sub> isopleth on the respective days as given by the Henderson and Prince method.

it is the case that lines drawn through pairs of points close to the equilibrium value pass close to the transection of lines drawn through pairs of points remote from this value. This demonstration we believe justifies the conclusion that the point of transection represents the true value of the tension of gases in the mixed venous blood.

Further support of this conclusion is given by a comparison of the tension of carbon dioxide in the mixed venous blood as determined by the present method and by the method of Henderson and Prince(6). It may be seen from the following table that the values obtained by

these two methods, when employed upon the same subject, agree to within about 1 mm.

Subject	Date	CO <sub>2</sub> tension of venous blood	
		Present method	Henderson and Prince method
A.C.R.	July 19	44.5 mm.	45.5 mm.
A.V.B.	" 8	49 "	49 "
"	" 7	49.5 "	50 "

The values which have been obtained by the method with resting subjects at sea level have fallen almost without exception between the limits of 50 mm. CO<sub>2</sub> and 31 mm. O<sub>2</sub> on the one hand and 45 mm. CO<sub>2</sub> and 36 mm. O<sub>2</sub> on the other. Between these limits for each millimetre fall in O<sub>2</sub> pressure there is approximately one millimetre rise in CO<sub>2</sub> pressure.

In practice we have adopted the following procedure for making the determinations. The gas mixtures are contained in a rubber bag of 5 litres capacity provided with a neck of rubber tubing 8 inches in length and  $\frac{3}{4}$ -inch bore. The neck is fitted with a glass mouth-piece behind which it may be closed off by a large pinch cock. Behind the pinch cock are two side tubes of small bore to which gas sampling vessels are attached (7).

In making the determinations the subject, who has been sitting at rest for 15 minutes or more, puts on a nose clip: empties his lungs as completely as he can, takes a deep rapid inspiration from the bag: exhales it rapidly into the bag: inspires deeply again, holds the inspiration for a second; then empties his lungs to mid-capacity into the bag. As this is accomplished the observer collects a sample of the air in the neck of the bag, which is the last portion expired. The remainder of the gas is held in the lungs 5 or 10 secs. longer, when an exhalation as deep as possible forces it into the bag and again a sample is taken. The results of the analysis of these two samples define the position of one line on the diagram. In working at sea level the determination is first made with the bag filled with nitrogen. This will give, when mixed with the residual air in the lungs, a pressure of O<sub>2</sub> equivalent to, or slightly less than, that of the venous blood, and a CO<sub>2</sub> pressure decidedly less. A second determination is then made with the bag filled with a mixture in which about 5 p.c. of O<sub>2</sub> is added to the nitrogen. This will give a sample having a higher O<sub>2</sub> and lower CO<sub>2</sub> pressure than the venous blood. A third determination may be made with a similar mixture to which 6 or 8 p.c. CO<sub>2</sub> has been added in order to obtain samples having a CO<sub>2</sub> pressure equal or greater than the venous blood. In practice it is

simpler, and justified by experience to determine the  $\text{CO}_2$  tension by the method of Henderson and Princee and draw a horizontal line on the diagram representing this isopleth

In using the method at high altitudes, the procedure is modified only to the extent that rather more oxygen is added to the gas mixture in the bag, to compensate for the lower pressure of  $\text{O}_2$  in the residual air of the lungs. The feasibility of doing this makes the method particularly well suited to work under anoxic conditions

There are some conditions, not yet accurately determined, which invalidate the result obtained by any method. Although the method given above is difficult for the subject to carry out, it has the great advantage, that a worker is in no doubt whether he can accept his result or not. If three or more lines run through, or close to, the same point, the result can be accepted. If they do not, the result must be rejected. The method we consider is accurate to about 1 mm. for each gas tension

A portion of the expenses of this research has been borne by the Proctor Fund, Harvard, the remainder by the funds of the Peru High Altitude Expedition

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ON THE CONCENTRATION OF THE BLOOD AND  
THE EFFECTS OF HISTAMINE IN ADRENAL  
INSUFFICIENCY. BY C. H. KELLAWAY (Foulerton  
Student of the Royal Society) AND S. J. COWELL.

(From the Medical Unit, University College Hospital Medical School.)

THE experimental production of acute adrenal insufficiency by removal of both glands is attended, as Gradinescu<sup>(5)</sup> first showed, by a steady rise in the number of red corpuscles per c.mm. of blood. Donath<sup>(3)</sup> subsequently observed that the weight of the dry residue of the blood increases after bilateral extirpation. Dale<sup>(1)</sup> also observed this concentration of the blood after removal of both adrenals and found associated with it a greatly increased susceptibility to histamine.

Our investigations have been primarily directed to determining the parts played by defect of cortex and medulla respectively in causing this concentration of the blood and increased susceptibility to histamine. In the course of our work we have noted certain other features of adrenal insufficiency, and investigated the concentration of the blood caused by injecting intravenously small doses of histamine.

#### METHODS.

The experiments were made on adult cats<sup>1</sup>. In order to destroy the medulla, radium emanation, in dose generally varying from .26 to 1.5 mg., was embedded in it. The emanation was enclosed in a sealed glass capillary tube 4 to 6 mm. in length, and just under 1 mm. in diameter. The adrenal was exposed through the flank, slung up by a ligature tied to the divided lumbo-adrenal vein, and a small incision made into one pole. The tube containing the emanation was placed in the end of a glass tube bent nearly to a right angle and just large enough to contain it. The containing tube was thrust into the medulla, and the emanation tube was extruded by a piece of flexible piano wire, prevented from going too far by a glass bead. Since the emanation has a rather rapid rate of decay it was left in the gland, except in a few cases in which larger doses (15 to 21 mg.) were implanted, for four or more hours and removed at a subsequent

<sup>1</sup> All survival operations were carried out by Kellaway under ether anaesthesia with full aseptic precautions.

operation. The amount of necrosed tissue was investigated at death by means of serial sections stained by Kohn's method (8). The medulla was chiefly affected, but a variable amount of cortex was involved as well, according to the position and amount of the emanation and the size of the gland. There was no evidence of any selective action on either cortical or medullary tissue. In some experiments, instead of sectioning the gland at death the amount of adrenaline remaining in it was estimated by Elliott's method (4).

To determine the amount of medulla remaining at different periods during life, we have noted the degree of paradoxical pupil dilatation produced by anoxæmia and by injecting histamine. The justification of this needs somewhat detailed treatment and we deal with it in a separate section. Injections of adrenalinic to standardise the reactions of the pupil, and of histamine, were made into an ear vein, the animal resting quietly on the knees of one of the observers during the injection. Anoxæmia was produced by causing the animal, which was gently held on its side, to inhale pure nitrogen for 45 to 60 seconds from a mask similar to that

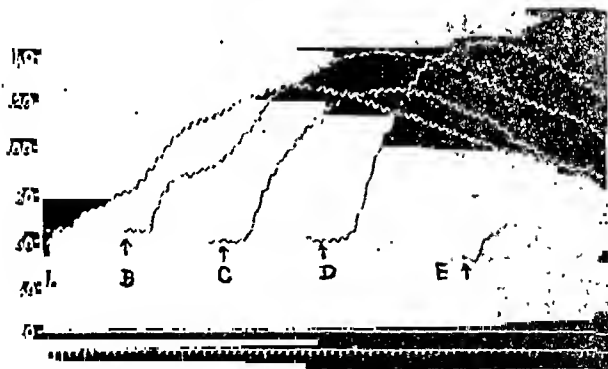


Fig. 1. Cat: Brain and most of spinal cord destroyed, then abdominal viscera removed.  
Blood-pressure tracing.

- A. Left splanchnic stimulated, coil at 18 cm.
- B. " " " " 10 "
- C. Adrenaline chloride .007 mg. injected
- D. " " .01 " "

Left adrenal excised.

- E. Left splanchnic stimulated, coil at 10 cm.



described by Kellaway (7). The pupils were examined under uniform illumination immediately after the injection or inhalation.

In some experiments, the amount of functional medulla remaining at the time of death was estimated by stimulating the peripheral end of the splanchnic nerve. In a cat with undamaged adrenals, which has been pithed and eviscerated, a large rise of blood-pressure is obtained either on stimulating the splanchnic nerve or on injecting adrenaline. After the removal of the corresponding adrenal a small rise only is produced by stimulation. Fig. 1 shows the results in such an experiment.

As an illustration of the application of the method we give Fig. 2. The blood-pressure tracing was taken from a cat (Exp. 1) in which .68 mg.

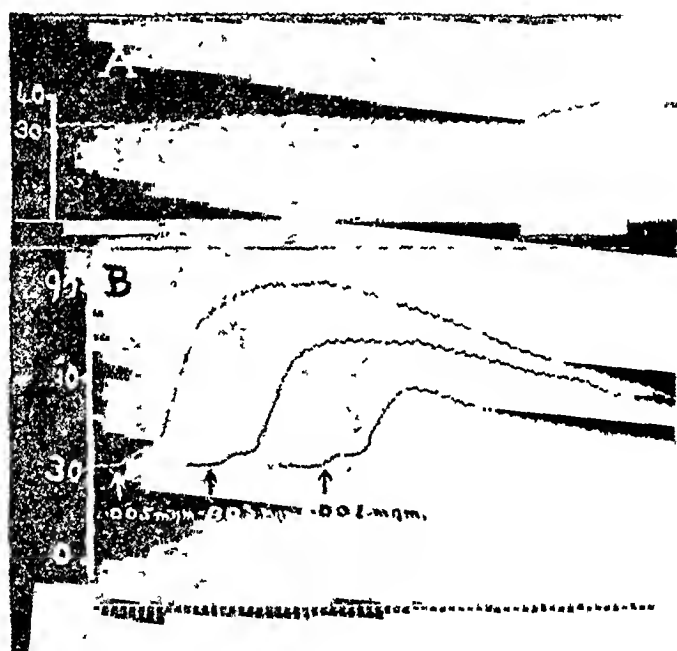


Fig. 2. Cat: Exp. 1, see text. Time in 2 sec. intervals.

- A. Stimulation of left splanchnic, coil at 18 cm. and at 10 cm.  
The right splanchnic gave a little less effect.  
B. Injection of adrenaline.

radium emanation had been implanted in the left adrenal, and the right removed five weeks later. Thirty-three days after this operation the cat was anæsthetised with ether, and after the brain and spinal cord had been pithed, was eviscerated.

The experiment shows the absence of functioning medulla, unless we

suppose that the emanation has a selective action on the nerve filaments to the gland of which we found no sign.

In order to destroy the cortex, the surface of the gland was seared with a small Paequelin cautery. The hilum and the areas near the poles where the arteries enter the gland were avoided so as to interfere as little as possible with the blood supply of the medulla, so that the whole of the cortex was not in any case destroyed.

The hæmoglobin percentage of the blood was determined with a Haldane's hæmoglobinometer, samples of blood being obtained by puncture of an ear vein. The changes in concentration which result from anoxæmia or from the injection of histamine were followed by taking successive samples of .05 c.c. of blood from an ear vein. These were diluted with 5 c.c. of distilled water to which a drop of ammonia had been added and after saturation with coal-gas, were compared in a Duboseq colorimeter.

After a short series of ranging experiments with emanation to obtain the approximate dose for medullary destruction, 12 experiments were made, one of which (Exp. 6) is given in the appendix. In the text, extracts from typical experiments are given, the experiments are numbered Exp. 1, Exp. 2, etc. in the order in which they are referred to, except when more than one extract is taken from the same experiment.

*Estimation of the amount of medulla in the adrenals by the paradoxical pupil dilatation. Effect of histamine.*

The superior cervical ganglion was removed on one side in order to induce a condition in which the paradoxical pupil dilatation could be obtained. It has been shown by one of us (7) that severe anoxæmia, produced by adding nitrogen to the inspired air or by inhalation of pure nitrogen, causes no paradox or only a trivial one in adult cats after the removal of the adrenals. We have found that the injection of histamine also causes little or no paradox in the absence of the adrenals. In the course of our experiments the following evidence was obtained as to the value of the reaction of the denervated pupil to histamine and to anoxæmia as a test of the amount of medulla which had escaped destruction.

In cats with intact adrenals, the degree of paradox produced by anoxæmia and by injecting histamine runs parallel with that caused by injecting adrenaline. Usually the results are as in Protocol 1.

Protocol 1. Cat, Wt 2.47 kilos. Right superior cervical ganglion excised under ether anaesthesia 12 days previously.

Adrenaline chloride, .001 mg.,  $\frac{1}{2}$  maximal paradox, lasting 25"; .002 mg.,  $\frac{3}{4}$  maximal, lasting 55".

Histamine, .005 mg., trivial, lasting 20": .01 mg.,  $\frac{1}{2}$  maximal, lasting 30": .02 mg.,  $\frac{1}{2}$  maximal, lasting 1': .05 mg.,  $\frac{3}{4}$  maximal, lasting 3'.

Nitrogen inhaled 30",  $\frac{1}{4}$  maximal, lasting 30": nitrogen 55", nearly maximal, lasting 3'.

But the amount of adrenaline required to produce a certain degree of paradox varies in different cats; it may take even .02 mg. to produce a paradox. When the adrenaline is less effective, so also are anoxæmia and histamine, as in the example Protocol 2.

Protocol 2. Cat, Wt 2 kilos. Pupil reactions 20 days after excision of the right superior cervical ganglion under ether anæsthesia, before interference with the adrenals.

Adrenaline chloride, .001 mg., nictitating membrane retracted, no trace of paradox; .004 mg.,  $\frac{1}{4}$  maximal, lasting 30".

Histamine, .01 mg., nil; .02 mg., nil; .04 mg., nil.

Nitrogen inhaled 40", nictitating membrane retracted, no paradox; nitrogen 55", doubtful transient pupil reaction.

The parallelism of the effects of anoxæmia and histamine on the one hand and of adrenaline on the other, ceases when the medulla of the adrenals is at all seriously decreased. In the experiment in which radium emanation was implanted in one gland and the other gland excised, the degree of paradox caused just before death by anoxæmia and by injecting histamine corresponded with the amount of medulla found post-mortem. The reaction to adrenaline, with one exception, was not altered by removal of the remaining healthy adrenal. The following will serve as an example of an experiment in which only a small amount of medulla remained.

Exp. 2. Radium emanation in both adrenals (1.3 mg. in right gland, .84 mg. in left). Animal killed four months later. The right gland was completely necrosed. The left gland contained a few chromaffin cells at one end only, a large part of the cortex being intact.

Three months after the implantation, when the cat was in excellent health, the reactions of the pupil to adrenaline remained unaltered, but the dose of histamine which earlier had caused a large paradox had no effect, and an inhalation of nitrogen for 50 seconds caused no pupil paradox though there was slight retraction of the nictitating membrane.

Further, the emanation has a gradual action on the gland and we find that the effect of anoxæmia and of histamine in animals that survive partial destruction of the adrenals, progressively diminishes till it ceases. Thus, in the experiment just mentioned both histamine and anoxæmia caused marked paradox a fortnight after the implantation, though less than before it (cf. also Exp. 6 in the Appendix).

The facts given above show conclusively, we think, that the degree of paradoxical pupil dilatation caused by anoxæmia and by histamine is a measure of the amount of functioning medulla remaining at the time. The mode of action of anoxæmia and of histamine is more controversial.

Following the previous conclusion of one of us (7) on the action of anoxæmia, we have taken the effect to be due to their liberating adrenalinic from the medulla. As regards anoxæmia this view has been contested by Stewart and Rogoff (10). They claim to have shown by their "vena cava pocket" method that asphyxia does not cause increased output of adrenaline. Their explanation of the action of asphyxia is that it "favours" the action of adrenaline so that pupil dilatation is produced without change in the amount of adrenaline in the blood. In the conditions of our experiments, however, severe anoxæmia causes little or no dilatation of the pupil in the absence of the adrenals. In consequence any "favouring" effect there may be seems to us to be too small to account for the great pupil dilatation that occurs when a moderate amount of adrenal medulla remains. Further, the amount of injected adrenaline required to produce a paradox is decreased little or not at all at a time when anoxæmia and histamine cause none, which does not suggest that the adrenalinic normally present in the blood is near the threshold necessary to cause the paradox.

One or two points may be noticed with regard to the action of histamine. Its first effect is to cause dilatation of both pupils and when large doses are given the dilatation is of some duration and may mask the paradox. The paradox produced by the injection of histamine in the cat with intact adrenals is not due to the fall of blood-pressure which causes the dilatation, since no paradox is produced in the absence of the adrenals although the blood-pressure falls. At Dr Dale's suggestion the action of acetyl choline was tried by one of us. In a cat which gave a paradox with .001 mg. of adrenaline and .008 mg. of histamine, .04 mg. of acetyl choline caused severe general effects with transient dilatation of both pupils but no trace of paradox. Histamine produces the paradox in cats with intact suprarenals after section of the splanchnic nerves on both sides. On our view that it does so by the liberation of adrenaline this effect must be due in part at least to direct action on the adrenals.

*The concentration of the blood in various types of insufficiency.*

In the experiments in which we implanted emanation in one adrenal and subsequently removed the remaining gland, we used a wide range of dosage. With the largest doses we obtained complete destruction of both cortex and medulla, with death from acute adrenal insufficiency soon after the removal of the second gland. With the smallest doses the damage was chiefly to the medulla, as was shown both by post-mortem examination and by the pupil reactions during life.

Some degree of concentration occurred in almost all the animals with varying degrees of insufficiency which we studied. In those which died rapidly, the rise in hæmoglobin percentage, the fall in body weight and temperature, and the slowing of the respiration rate proceeded side by side until death, and the hæmoglobin value was an excellent prognostic guide to the ultimate issue, as in the following experiment:

Exp. 3. Cat, Wt 1.93 kilos. .92 mg. emanation in left adrenal, right adrenal removed 42 days later. Wt 1.89 kilos. Hb. 75 %. Resp. 50 per min.

Days after removal of right adrenal	Wt in kilos	Hæm. %	Resp. per min.	Temp.
1	1.86	88	53	39.6
2	1.83	88	52	39.4
3	1.82	92	45	39.1
5	1.74	98	33	38.3
6	1.70	104	30	38.0
7	1.68	104	25	37.8
8	1.63	112	Slow, irregular	33.3

Animal exhibited twitchings and unsteady gait; blood-pressure under ether 78 mm., killed. In the left gland the cortex was nearly all destroyed, but a little remained at each pole, and there were only a few small areas of chromaffin tissue. The pupil reactions also showed that there was very little medulla.

In this animal the denervated pupil was slightly less sensitive after the final operation. It may be noted that this was the only animal in which any change in the sensitiveness of the denervated pupil was observed after interference with the adrenals.

The operation of removal of the second adrenal appears itself to cause a rise in the hæmoglobin value, which is probably due to the action of the anæsthetic. In cats dying rapidly there is no recovery from this post-operative rise and the hæmoglobin value continues to increase till death. In this cat the hæmoglobin had risen from 75 p.c. to 85 p.c. by the afternoon of the day on which the final operation was performed and was only 88 p.c. after 24 hours. This early abrupt rise was probably post-operative. In animals dying less rapidly this post-operative concentration is often more evident. After a rapid initial rise the hæmoglobin may remain stationary for one or two days or even fall slightly before resuming the steady increase which continues till death. This is illustrated by Exp. 4 in which, except for a small area of cortex at one pole, the whole of the left adrenal was destroyed after the implantation of 21 mg. of emanation for 18 hours, 15 days before the removal of the right gland. The animal survived 17 days after this last operation, finally dying with the usual symptoms of adrenal insufficiency. The hæmoglobin percentage before the removal of the second gland was 80 p.c., four hours after the operation

it had risen to 88 p.c., 24 hours after it was 100 p.c., 48 hours after it had fallen to 96 p.e. It then increased steadily, reaching 140 p.c. at death.

In cats dying with a more chronic type of insufficiency the concentration has a different character. Fig. 3 shows the changes in weight and

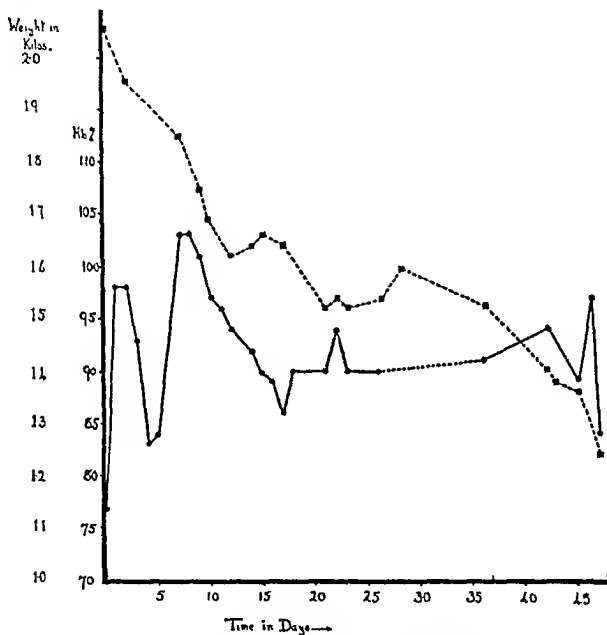


Fig. 3. See text. Interrupted line = change in body weight.  
Continuous " = " haemoglobin percentage.

in haemoglobin percentage in a cat (Exp. 5) which survived 48 days after removal of the right adrenal, the left gland having been treated by the implantation of a dose of .26 mg. of emanation two months previously. No accessory adrenals were found at autopsy and the left gland, which was a very small one, when examined by serial sections showed only a few isolated chromaffin cells, though a good deal of cortex remained. After the removal of the right adrenal the haemoglobin rose from 77 p.c. to 98 p.e., fell on the fourth day to 83 p.e., again rose on the seventh day

(the hæmoglobin before removal of the unimplanted adrenal was 67 p.c. and at death was 71 p.c.) a deep-seated stitch abscess was found at autopsy and we think the infection caused anæmia.

*The concentration of the blood following the injection of small doses of histamine in animals with adrenal insufficiency.*

The second concentration phenomenon which we investigated was that which follows the injection of small doses of histamine in cats with adrenal insufficiency. The changes in hæmoglobin percentage in three normal cats following the injection of doses of histamine ranging from .01 to .05 mg. were as follows:

Dose of histamine in mgs.	Hb. % before injection	Hb. % after injection				
		3'	10'	15'	20'	30'
(1) .01	52	52	—	—	—	—
	.02	55	—	—	—	—
	.05	60	—	—	54	52
(2) .01	85	85	—	—	—	—
	.05	94	88	84	—	—
(3) .01	75	83	75	—	—	—
	.05	85	79	75	—	—

These examples are typical of a large number of observations. It is seen that a dose of .05 mg. causes a rise of the order of 10 p.c. which has disappeared in most cases in from 15 to 20 minutes after the injection. Smaller doses may or may not cause any concentration.

A reaction of quite a different character occurs in cats with acute adrenal insufficiency. Fig. 4 shows the responses to three separate injections of .05 mg. of histamine in a cat (Exp. 9) in which the adrenal glands were removed at successive operations with an interval of nine days between them. *A* is the change in hæmoglobin percentage in response to an injection after the removal of one adrenal, but before the extirpation of the second. The hæmoglobin rose from 84 p.c. to 90 p.c., falling to normal in 20 minutes. After the removal of the second gland the cat survived three days. *B* and *C* are the responses to injections 24 and 48 hours respectively after the final operation. In *B* the rise is from 94 p.c. to 103 p.c., and the hæmoglobin has not returned to normal at the end of one hour. In *C* the rise is from 100 p.c. to 115 p.c. and two hours afterwards the blood is still more concentrated than before the injection. In chronic insufficiency similar changes were observed except that in the later stages the increase in concentration was much slower and lasted longer. Thus, in Exp. 5, .1 mg. of histamine injected on the 47th day caused no change in hæmoglobin percentage in 10 minutes. In

the following 20 minutes the concentration rose from 97 p.c. to 101 p.c. and in two and a half hours it was 108 p.c.

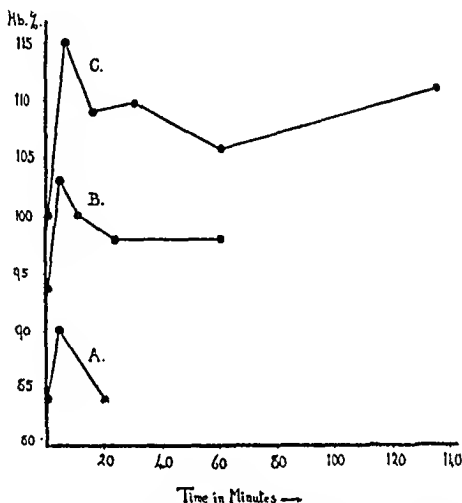


Fig. 4. Cat: Change in haemoglobin percentage caused by .05 mg. histamine. *A*, after removal of one adrenal. *B* and *C*, after removal of the remaining gland.

When the loss of medulla is much greater than that of the cortex, the haemoglobin percentage rises rapidly and remains high for a considerable time. Thus, in Exp. 6, 71 days after removal of the untreated gland, the percentage rose from 87 to 99 in the first four minutes after injecting .05 mg. of histamine, remained at this level for 15 minutes, fell to 93 in the next hour, but had not returned to normal in three and three-quarter hours. And in another experiment (Exp. 7) in which a large amount of medulla was destroyed but less cortex, histamine had much the same effect, but the rise in haemoglobin percentage did not last quite so long, returning to normal in two and a half hours.

On the other hand, when the cortex is largely destroyed by cauterisation and the medulla is relatively little affected the rise in the haemoglobin percentage is much like that of a normal cat. Thus, in Exp. 10, .05 mg. of histamine on the third day after removal of the untreated gland caused



in four minutes a rise from 126 p.c. to 137 p.c., but this returned to normal in 15 minutes.

The facts given above show that the type of concentration caused by small doses of histamine are due to medullary and not to cortical defect. This conclusion is reinforced by a further observation which we have made concerning the concentration in response to histamine, both in normal cats and in those with varying degrees of insufficiency. This concentration either does not occur at all or is of a very trivial character if .5 to 1 c.c. of a .1 p.c. solution of adrenaline chloride is injected subcutaneously and given time to produce a paradoxical pupil reaction. The pupil reaction usually appears 10 to 15 minutes after the injection and lasts for four or five hours. The following are examples of the changes in the histamine concentration reaction of three normal cats caused by the previous injection of adrenaline. The hæmoglobin percentage is estimated before and at various periods after the injection of .05 mg. of histamine.

Relation to adrenaline injection	Hb. % before injection of histamine	Hb. % after injection of histamine			
		3'	15'	20'	30'
(1) Before	85	94	84	—	—
10' after	85	85	85	—	—
(2) Before	75	85	79	75	—
40' after	75	75	—	—	—
(3) Before	52	60	55	—	52
97' after	52	55	—	—	—

The same effect is produced in the case of the histamine concentration reactions in cats with adrenal insufficiency as the following examples show:

Exp.	Relation to adrenaline injection	Hb. % before histamine	Hb. % after injection of histamine				
			4'	15'	30'	1 hr.	2 hrs.
6	Before	87	101	—	—	92	—
	10' after	87	92	87	—	—	—
1	Before	80	95	91	88	83	—
	12' after	80	79	80	—	—	—
5	Before	101	112	112	111	111	119
	5 hrs. after	103	104	102	—	—	—

In the normal animal the effect of histamine is to cause a concentration which quickly disappears. The evanescent character of this effect seems to be due to the antagonistic action of adrenaline put out in increased amounts as the result of the injection. In animals with medullary insufficiency the absence of adrenaline in sufficient amount in the circulation to antagonise the injected histamine allows the concentration to last for a longer period. Anoxæmia causes a concentration similar to that

produced by histamine. We have not investigated this phenomenon in any detail.

*The hypersensitiveness of animals with adrenal insufficiency to histamine.*

Closely related to the histamine concentration effect is the increased sensitiveness of cats with adrenal insufficiency to the toxic effects of histamine. Dale<sup>(1)</sup> found that 10 mg. per kilo or even larger doses of histamine could be administered with only transient toxic effects to normal non-anæsthetised cats by slow perfusion of a .1 p.c. solution. On the other hand animals deprived of their suprarenals, before the appearance of any obvious signs of adrenal insufficiency, died as the result of the infusion of amounts varying from .16 to .75 mg. per kilo. In our experiments we studied these toxic effects of histamine in a different way, though in one or two experiments we used Dale's method of infusing histamine into the saphena vein. A dose of .1 or .05 mg. of histamine in .01 p.c. solution in saline injected into the ear vein of a normal cat causes some of the symptoms observed by Dale and Laidlaw<sup>(2)</sup> following the injection of larger doses. There are transient wide dilatation of the pupils, inhibition of the heart, sweating of the pads, salivation and congestion of the mucous membranes. There is never dyspnoea, nor is the circulatory failure so marked as to cause even the transient collapse of the animal.

A very different picture is observed after the injection in animals suffering from adrenal insufficiency; the cat walks a few steps and after about 30 seconds falls over on its side with severe inhibition of the heart, pronounced dyspnoea and sometimes with Cheyne-Stokes respirations. This collapse is in some cases attended with general convulsions: the pupils are widely dilated and the animal looks as if it were about to die. However, in a variable time, four to ten minutes, it gets up and walks away apparently recovered.

Exp. 9. A cat, weight 1.8 kilo, in which the adrenal glands were completely removed at two successive operations with an interval of a week between them, died of acute adrenal insufficiency three days after the second operation and no accessory adrenals were found at autopsy. Twenty-four hours after the removal of the second gland .05 mg. of histamine injected intravenously caused severe dyspnoea which lasted for some minutes, but no collapse. Forty-eight hours after the operation, when weakness and lowering of the body temperature were evident, the same dose of histamine caused convulsions and collapse in a few seconds; the cat remained in a condition of profound shock with dyspnoea for a few minutes and then slowly recovered.

We have studied the results of such small injections of histamine in cats with varying degrees of insufficiency. The gradual appearance of this

abnormal type of response to histamine in chronic adrenal insufficiency is well illustrated by Exp. 5 in which the cat survived 48 days after removal of the untreated gland. Before the extirpation of the second gland an infusion by Dale's method of 10 mgs. of the drug per kilo produced only the transient effects observed by Dale in normal animals and small doses failed to cause any symptoms of collapse. Nine days after the extirpation of the untreated gland .1 mg. of histamine caused shock and severe dyspnoea. This hypersensitiveness to histamine continued throughout the life of the animal and increased towards the end.

In cats surviving after extensive destruction of the medulla of one gland and complete extirpation of the other, these toxic effects were not constantly observed. In Exp. 6 this type of response occurred in the cat for the first time a fortnight before its death. The animal in Exp. 1 showed increased sensitiveness to .05 mg. of histamine throughout its period of survival, but that of Exp. 7 at no time gave reactions of this type. In Exp. 2 in which emanation was embedded in both glands, the animal during the four months which elapsed before its death, showed no trace of this hypersensitiveness, though the infusion of 6 mg. per kilo of histamine caused its death some hours after the injection.

Animals in which the cortex of one gland was severely damaged without corresponding injury to the medulla almost invariably showed, after the extirpation of the untreated gland, hypersensitiveness to small doses of histamine. In Exp. 11 the right adrenal was removed 14 days after the cauterisation of the left gland. The animal remained in good health and was killed four and a half months later; a mass of cortical tissue, half the size of a normal gland, had developed at the site of the ligature on the right adrenal vein. During the first two weeks after the removal of the untreated gland, the animal showed extreme general effects following the injection of .05 mg. of histamine. The reaction became later less evident though the medulla showed no corresponding increase in function as judged by the reaction of the denervated pupil.

The symptoms produced by small doses of histamine in hypersensitive animals are usually profoundly modified by previous subcutaneous injection of adrenaline. Excess of adrenaline in the circulating blood antagonises the histamine and prevents collapse from occurring. Schenk (9) has also found some evidence of this antagonistic action in man. In view, however, of the results just described we cannot avoid the conclusion that deficiency of the cortex plays an important part in the hypersensitiveness to histamine of animals with adrenal insufficiency. The antagonistic action of the subcutaneous injection of adrenaline may

be to raise the blood-pressure to such a level as to make collapse impossible. The increased output of adrenaline, which we consider histamine causes, would serve to protect the animal against histamine collapse.

*The symptoms of chronic adrenal insufficiency.*

Our experiments, so far as they go, support the conclusions of Swale Vineent and Wheeler(11) and of others that animals can survive in apparently good health for many months in the absence of nearly all the intra-medullary chromaffin tissue. Further, the destruction of sufficient cortex appears to cause symptoms of acute insufficiency while some of the medulla is still functioning, though as the symptoms of insufficiency become more marked the output of adrenaline from the residual medulla can no longer be demonstrated by the paradoxical pupil reactions.

There is very little in common between the symptoms of Addison's disease and the picture presented by animals dying rapidly of adrenal insufficiency, apart from the lowered blood-pressure and muscular weakness which appear in both. The symptoms which appear in animals which survive in good health for long periods after the destruction of most of their intra-medullary chromaffin tissue are not very striking. The most notable change is in the temperament of the animals, which become quieter and more subdued. A subcutaneous injection of .5 mg. of adrenaline generally restores the animal for a time to its normal pre-operative condition. One animal which was rather wild before operative interference with its adrenals became thoroughly tame, but reverted to its wild state after the injection of adrenaline.

Another symptom which we observed, though in two animals only, was the development of dark brown patches of pigment in areas of the skin which had been shaved. One (Exp. 6) was a black cat, the other was a tabby (Exp. 7). The skin when first shaved had a pinkish-white colour, but when shaved again a month later the general ground colour had become a dark grey and in addition there were definite patches of brown pigment scattered over the area. Control animals treated in the same way showed the general darkening of the skin but there were no patches of brown pigment. In none of our cats did we observe any pigmentation of the mucous membranes. The brown patches of pigment in these experimental animals were shown by section to be iron-free. The pigmentation was therefore non-hæmatogenous. The result is suggestive of a possible relation between the pigmentation observed in Addison's disease and the destruction of the medulla of the adrenals.

The destruction of the medulla may lead to the formation of non-hæmatogenous pigment from some precursor of adrenaline which cannot be elaborated by the damaged gland as in the normal animal.

In chronic insufficiency of both cortex and medulla terminating in death the symptoms resemble more closely those occurring in acute experimental insufficiency than those met with in Addison's disease. The animals usually appear to be in fairly good health until shortly before death. There is gradual loss of weight, increasing muscular weakness and change of temperament, but lethargy appears only with the fall of body temperature and slowing of the respiration rate which precede death. Towards the end a very striking symptom in animals dying from either acute or chronic insufficiency is the frequent twitching of the skeletal muscles. This can be abolished by placing the animals in a heated cage; the rectal temperature rises a few points, and the twitchings, which are probably a gross form of shivering, disappear, to reappear later if the animals are removed from the source of heat.

The rise in hæmoglobin percentage is an early and valuable sign of insufficiency. The injection of small doses of histamine also provides a useful indication of the progress of insufficiency.

#### CONCLUSIONS.

(1) The paradoxical pupillary reactions to anoxæmia and to the injection of small doses of histamine are a useful guide to the amount of functioning medulla in animals with varying degrees of adrenal insufficiency.

(2) Histamine causes increased output of adrenaline and the effect is in part a direct one on the adrenals.

(3) The concentration of the blood in animals dying of adrenal insufficiency is due to cortical defect.

(4) The altered concentration reaction to small doses of histamine in such animals is due to medullary defect.

(5) Both cortical and medullary defect appear to play a part in the "collapse" type of response to the injection of small doses of histamine.

(6) The subcutaneous injection of adrenaline antagonises the action of histamine in causing concentration of the blood. It also masks the hyper-sensitiveness to histamine exhibited by animals with insufficiency.

The emanation used in these experiments was supplied to us free of charge by the Radium Institute. We wish to express our indebtedness to Mr Alton, the Director of the Physical Laboratory of the Institute, and to Dr Eugene Wassmer, who prepared the emanation tubes for us.

*Appendix*

Exp 6 Cat, weight 2.2 kilos

2 ix 21 14.8 mg radium emanation embedded in left adrenal, removed four and a half hours later

3 x 21 Extirpation of right adrenal under ether anaesthesia

9 xi 21 Excision of left superior cervical ganglion under ether anaesthesia

25 vi 22 Death from enteritis Up till a few days before this event the animal was in good health, its weight was 2.2 kilos and the only changes noted apart from those in the pupil reactions were its alteration in temperament, the cat having become very much quieter than it was before interference with its adrenals, the development of dark brown patches of pigmentation in shaved areas of the skin, and certain changes in the concentration of the blood

The pupil reactions, which were tested many times during this long period of survival, showed extreme diminution in the output of adrenaline in response to anoxæmia and to the injection of histamine. On no occasion could any trace of paradox be obtained with histamine, though sometimes a barely perceptible reaction was observed in response to anoxæmia, the pupil remained throughout extremely sensitive to adrenaline. The following are examples of the pupil reactions during this period

Adrenaline chloride 0.025 mg, nearly maximal, lasting 99" 0.01 mg,  $\frac{1}{2}$  maximal, lasting 39", 0.005 mg, perceptible paradox, lasting 39"

Histamine 0.2 mg, nil, 0.4 mg, nil, 1 mg, nil (reaction may have been masked by prolonged dilatation of both pupils)

29 xi 21 Nitrogen inhaled 50"—just perceptible paradox, lasting 2

3 xii 21 Nitrogen inhaled 45"—just perceptible paradox, lasting 2

16 i 22 Nitrogen inhaled 70"—no trace of paradox

Nitrogen inhaled 55"—perceptible paradox, lasting 99"

12 vi 22 Nitrogen inhaled 55"—no trace of paradox

For other results compare pages 90, 93, 94, 96 and 97

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# ON THE NATURE OF THE SUGAR IN BLOOD.

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THE experiments of Minkowsky<sup>(1)</sup> first established the importance played by the pancreas in the metabolism of carbohydrate. Clark<sup>(2)</sup> showed that the consumption of sugar by the heart was increased when the perfusing fluid was also passed through the pancreas. In a later paper<sup>(3)</sup> he established the remarkable fact that perfusion of the pancreas with Locke's solution, *i.e.* one containing dextrose caused the optical rotation to be diminished whereas the copper reducing value remained unaltered. He suggested that the pancreas contained an enzyme which was responsible for converting dextrose into another form of sugar, which then could be utilised by the body.

The recent work of Embden and his co-workers<sup>(4)</sup> has emphasised the importance of hexose-phosphoric acid in the carbohydrate metabolism of muscle. Since on hydrolysis hexose-phosphoric acid yields fructose, it seemed possible that the sugar in normal blood might be other than glucose. The primary aim of our experiments—begun in 1921—was, then, to investigate the nature of the sugar in the blood by a comparison of the polarisation, and copper reducing values. For this it was necessary to prepare a protein-free filtrate.

## *Method.*

After testing the various protein precipitation methods usually employed for blood sugar estimations, we found that they all gave on concentration a fluid which was quite unsuitable for polarimetric determination. The tungstic acid method of Folin and Wu<sup>(5)</sup> was least tedious, and gave a filtrate which was almost free from the precipitant; that all protein was not removed was made clear by nitrogen determinations, and by the turbidity which appeared when the filtrate was concentrated to a small bulk. It was necessary, therefore, to employ some further process in order to remove the remainder of the proteins and to extract the sugar. The use of alcohol in a suitable strength to effect both processes simultaneously suggested itself. In a series of trials 85 p.c.

alcohol was found to be the best extraction medium; this strength extracts all the sugar likely to be present and is sufficiently strong to coagulate all the remaining proteins. Until a neutral sample of sodium tungstate was obtained care was taken to adjust the reaction in the way recommended by Folin (6). Neutral samples were finally obtained from Messrs Harrington.

The method finally adopted is as follows: The blood is precipitated according to the method of Folin and Wu. Filtration is effected by filtering in succession through two funnels. In the upper one is placed a coarsely grained paper, in the lower a Whatman paper No. 40. The double filtration removes all of the precipitate which it is possible to remove by filtration at this stage. The filtrate is concentrated almost to dryness *in vacuo*, not allowing the temperature to rise above 40° C. Caprylic alcohol is used to prevent foaming. A volume of 85 p.c. alcohol equal to the original volume of blood is then added to the distillation flask, and an immediate precipitation of the remaining proteins and tungstate takes place. The alcohol is allowed to stand in contact with the residue thus formed at 40° C. for 20 minutes with occasional shaking, so as to ensure complete extraction of the sugar from the solid mass. The alcohol is filtered into a small distillation flask and a further quantity of alcohol equal to half the original amount is added to the first flask in order to complete the extraction. This is allowed to stand for ten minutes with shaking as before. The combined filtrates are concentrated *in vacuo* at 40° C. until almost dry. The residue is taken up in a small quantity of water, the amount depending upon the original volume of blood used, and then filtered (usually 20 to 30 c.c.). The filtrate is perfectly clear and suitable for polarimetric determinations. A one decimetre tube is usually employed owing to the amount of fluid available being small, and a considerable amount being required for the copper reducing estimation.

The final filtrate was tested by the protein and biuret colour reactions; negative results were obtained. A determination of the nitrogen showed that it was no greater than could be accounted for by the extraction by the alcohol of urea and similar substances. In order that an accurate comparison may be made between the polarimetric and copper reducing values of the blood sugar, it is necessary that every trace of protein be removed; if this condition be not obtained, not only are the optical properties of the solution liable to be affected to an unknown and variable extent in each experiment, but in determining the copper reducing value of the sugar, there is a danger of traces of protein giving rise to substances



which will reduce the alkaline copper solution. Owing to the careful control of temperature, and the very small concentration of acid, we do not consider that the method would tend to split off copper reducing substances from possible gluco-genetic bodies.

To determine how completely the sugar was extracted by the alcohol employed, the residue left behind after the filtration of the alcohol was shaken up and extracted with water. The filtrate now was perfectly clear showing that the remaining protein was not readily redissolved. Copper reducing determinations and polarimetric readings showed no measurable amount of sugar present. To test whether the relation between the polarimetric and copper reducing powers of glucose would be altered by this treatment, a solution of pure glucose was concentrated in the presence of tungstic acid employed for precipitation, extracted with alcohol, and made up with water. The copper reducing power was then, if anything, slightly lower than the polarimetric value. Possibly the larger amount of free acid caused slight disaccharide formation(7); normally all but a small amount of tungstic acid combines with the protein and is so removed.

The filtrate is invariably slightly acid and therefore most likely to stabilise the sugar originally present. It soon however became evident that the instability of the original sugar made it essential that concentration of the aqueous filtrate should be effected as quickly as possible. This we have always done, and as soon as the final filtrate was obtained we have made a quantitative comparison of the sugar by polarimeter and copper reducing power. We have made no attempt to obtain in our final filtrate all the sugar originally present in the blood. To have attempted quantitative extraction would have lengthened the process very considerably. Our sole concern was to obtain the sugar in a form as nearly as possible approximating to that normally in the blood. Our experiments from the beginning of protein precipitation to the polarimetric reading usually occupied five hours for 100 c.c. blood; when less blood was used the time taken was much less. Of this time only a comparatively short period is occupied in what we find to be the dangerous stage, viz. the concentration of the aqueous filtrate.

The copper reduction method which has been adopted throughout is that of Bertrand as modified by Wood and Berry(8); it had been previously determined by us that this gives reliable and consistent results; the large amount of fluid used and the convenience of a permanganate titration being points in its favour. A comparison of this method with values obtained from the polarimeter was undertaken with

a sample of pure glucose (for this we are indebted to Dr C G L Wolf) The degree of accuracy obtained may be seen from the following figures

Glucose weight taken	2435 gm	made up to 100 c c with water
Copper reduction value	23 %	
Polarimeter	242 ..	

The polarimeter used is a three field instrument made by Hilger of London, the illumination being the mercury green line This has the advantage of giving a higher specific rotation than that obtained from the sodium light The light also is steady and can easily be varied in intensity by the resistances with which it is connected By means of the instrument used readings to .01 degree can be obtained The mean of a series of readings by each of us was taken whenever possible As a rule complete agreement was obtained, it was noticeable though that when one of us had been bled this agreement no longer held, in these cases the reading from the other was accepted It had been previously noticed that a comfortable position and normal mental state are necessary for reading colorimeters and similar instruments

The amount of sugar extracted is approximately  $\frac{1}{3}$  to  $\frac{1}{2}$  of that indicated as being present by the micro estimation There is no possibility of more than one sugar being present and of one being preferentially extracted by the solution of alcohol employed, as no trace of sugar was found to be extracted by water after the alcohol treatment had been completed From the final filtrate in some of our experiments an osazone was prepared This was recrystallised Microscopic examination showed only glucosazone to be present, melting point determination gave  $205^{\circ}\text{C}$ , which was sharp and well defined This figure corresponds to the accepted value of the melting point of glucosazone

The figures obtained by Bang's old method (this was used by us for the quantitative estimation of the blood sugar content) give only an approximation to the actual content in the whole quantity of blood drawn owing to the sample being obtained at one period only of the operation This should be noted in connection with the nervous factor which is apparent in one of the cases discussed below

### *Experiments with animal blood*

A series of experiments was carried out using the blood of different animals, ox, sheep, cat and rabbit That from the ox and sheep was obtained from the slaughter house, that of the other animals, from those killed for the purpose in the laboratory without anæsthetics 25 to 100 c c were usually employed, depending upon the animal This blood

was treated as has been described above. The final filtrate when read in a one decimetre polarimeter tube usually gave a value considerably below that obtained by the copper reduction method. The copper reduction value was calculated as glucose. Calculating also the polarimeter reading as glucose the difference between the two results was very apparent.

The work of Hewitt and Pryde<sup>(9)</sup> suggested that an unstable form of glucose might be present; the tubes were therefore read for at least three days after the completion of the experiment. The readings in each case gradually approached, and usually reached at the end of this time, a rotation corresponding to the  $\alpha$ ,  $\beta$  equilibrium form of glucose. The copper reducing value was unaltered at the end of this time. As mentioned above, the speed at which the concentration of the aqueous filtrate is carried out is of the greatest importance. If faulty water-pressure delays the concentration, or the temperature is allowed to rise unduly, the polarimetric is very close to, or even above, the copper value. It was found that rapid concentration was most readily effected by employing large distilling flasks of two litre capacity. The distillation was begun with only about one half of the filtrate, the remainder being added at intervals by means of a funnel connecting with the capillary. The effect of this is to enable the distillation to proceed smoothly and continuously while the fluid is being added, and at the same time avoids the necessity of allowing air to enter the flasks in order to stir up the fluid.

To test whether appreciable alteration in the state of the sugar took place at room temperature when in contact with the precipitated proteins, two samples of the same blood were taken: one was precipitated, filtered immediately, and proceeded with as usual; the other was precipitated, allowed to stand for six hours, and then filtered and treated as usual. The readings of the final products were both identical as regards their copper reducing and polarimetric values.

Some experiments were carried out to determine whether a ferment or ferments are present in blood which will convert added glucose to another form of sugar. The sugar was added before and after precipitation of the proteins and allowed to stand at room temperature, 20° C., for one hour. The amount of glycolysis being inappreciable under these conditions, almost identical results were obtained from the two samples, indicating that no such ferment can be present, or at any rate, that it is unable to work under the conditions of our experiments. Hewitt and Pryde's experiments<sup>(9)</sup> suggested that if any change were to take place

it would occur with great rapidity. Our negative results are in agreement with the conclusion of Cooper and Walker<sup>(10)</sup>, who state that no such enzyme is present in the blood.

The only experiments which we can find in which the sugar of the blood has been investigated as regards its copper reducing power and its polarimetric value are those of Oppler<sup>(11)</sup>. We attribute the little or no difference he found in the two values to the long time required to obtain a concentrated protein free solution by the method he employed, and to the sugar being in aqueous solution throughout—conditions which would allow the sugar to be converted into the  $\alpha$ ,  $\beta$  equilibrium form.

#### *Experiments with human blood.*

In view of the results obtained from the blood of animals, it became of interest to determine the condition of the sugar circulating in the blood of man. The blood was drawn from an arm vein by means of a hypodermic needle and syringe into a known amount of .1 p.e. ammonium oxalate solution in a measuring cylinder, with constant shaking to prevent clotting of the blood. The precipitation was then carried out at once. These operations were carried out at Addenbrooke's Hospital, except in two diabetic cases. At the same time a sample of blood was taken on which a quantitative estimation was effected by Bang's old method.

In normal persons with one exception the first polarimeter reading on the final filtrate showed that sugar in the blood had a specific rotation which was considerably below that of  $\alpha$ ,  $\beta$  glucose as deduced from the copper value. This is entirely comparable with our experiments with the blood of animals. The curves approach the copper value in a similar fashion; in some cases this value was not reached, and some subsidiary action was evidently occurring. In Fig. 1 are shown the curves of the polarimetric readings of three normal individuals. It will be seen that in each case they start below their respective copper values. These are calculated for glucose. The curve of H.M. did not quite reach its end point in four days. Both of the others were in equilibrium on the third day.

Hewitt and Pryde's experiments<sup>(9)</sup> suggest that solutions of glucose and fructose in contact with the mucous membrane of the intestine are converted into  $\gamma$  glucose; presumably they enter the blood in this form; to test this it seemed desirable to determine whether any alteration in the nature of the blood sugar could be brought about by ingestion of considerable quantities of these sugars. While the experiments with glucose might be open to doubt on the grounds of a small difference in

the curve being possibly produced by delay in the concentration of the filtrate, any considerable amount of fructose present in the blood would

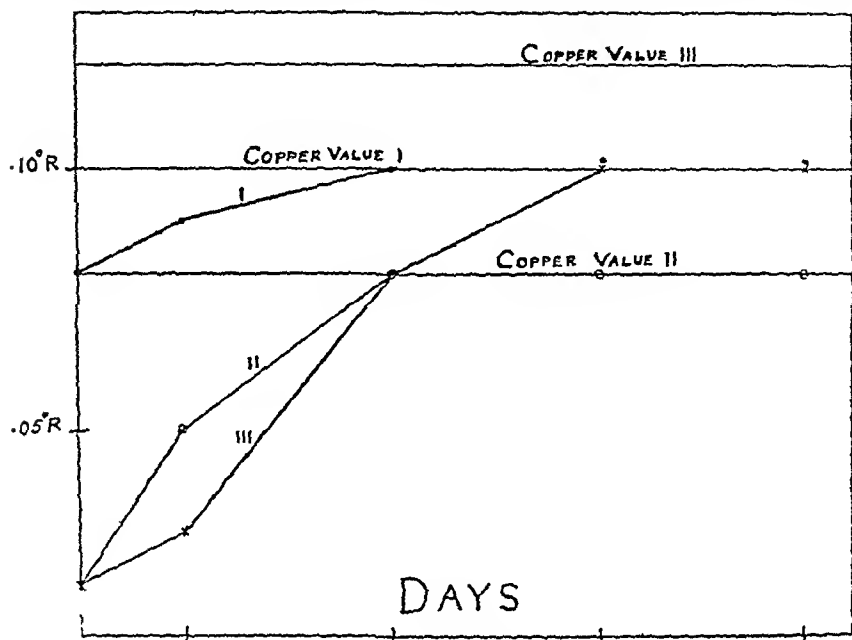


Fig. 1. Increase of polarimeter reading on successive days.  
Normal subjects: I. J. B. S. H. II. G. M. D. III. H. M.

be more likely to be detected, since the specific rotation of fructose is so much greater than that of glucose, and still greater than that of the normal sugar of blood. It might reasonably be expected that with fructose lævo rotations would be obtained.

The normal blood sugar content which we obtain by Bang's old method is  $.08$  p.c. to  $.1$  p.c. After meals of 100 to 150 grams of glucose or fructose this rises to  $.13$  or  $.14$  p.c., i.e. the content of the blood sugar has increased by 50 p.c. The blood was drawn half-an-hour after the meal. If this sugar had been in the condition as ingested, a marked alteration of the ratio copper reduction to polarimeter should have been apparent. This however did not occur. The ratio of copper reducing power to polarimeter reading was similar to that of normal blood. We conclude therefore that glucose and fructose enter as, or at any rate are very rapidly converted into, the form normally present in the blood. In Fig. 2 the results of three experiments on L. B. W. are shown. Quantities of blood varying from 55 to 100 c.c. were taken, but for purposes of comparison one copper line is taken in order to emphasise the fact that the ratio copper value to polarimeter was approximately the same

at the beginning of each curve. This shows that the sugar which recently entered from the intestine had been converted into normal blood sugar.

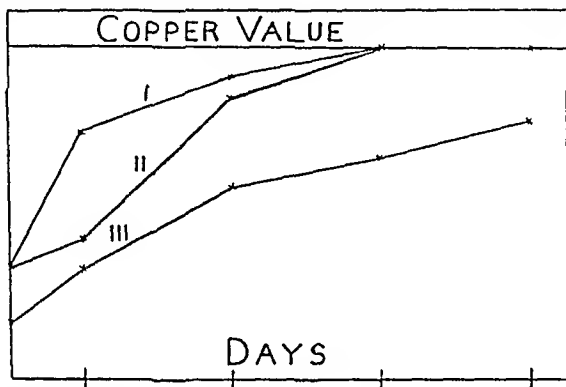


Fig. 2. Increase of polarimeter reading on successive days. L. B. W. I. Normal. II. After 100 grams of glucose. III. After 160 grams of fructose. (The curves are scaled to a common copper value.)

In view of the facts mentioned above, it became of interest to see whether the blood of patients suffering from diabetes mellitus differed from that of normal persons. The cases investigated of subjects suffering from diabetes have all been of the severe type. As a consequence it has only been possible to obtain a comparatively small quantity of blood from each. It is important to note that owing to the high sugar content a much smaller quantity of blood is required with a consequent acceleration of the manipulation. If the blood sugar was even approximately normal in quality it should be very evident, since there would be less time elapsing between the precipitation and the final product. In three cases the polarimeter reading was slightly above the copper reducing value. Any considerable amount of  $\beta$ -oxybutyric acid present in the final filtrate would tend to throw the rotation in a laevo direction, as the l-form is the only one present in these cases. The curves reached the copper value on the second or third days. In one case the polarimeter reading was considerably above that of the copper reducing value; the possible meaning of this is discussed later.

It is probable then, that in severe cases at any rate the normal blood sugar is present only in very small amounts and it is possible that the failure to utilise sugar is due to the lack of some ferment, which has

the property of converting glucose and fructose to the form normally present in the blood, and that the sugar in cases of diabetes is mainly the  $\alpha$ ,  $\beta$  equilibrium form of glucose.

In Fig. 3 curves are shown of two diabetic cases with one normal for

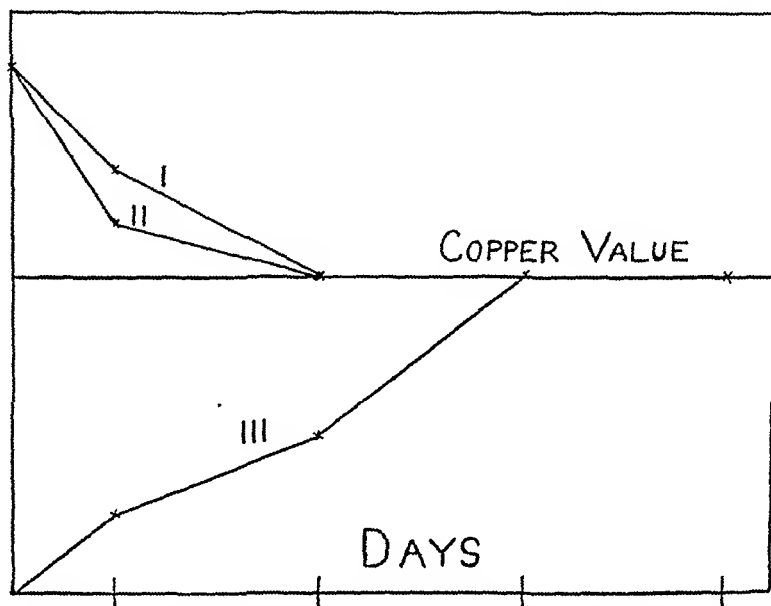


Fig. 3. Curves of polarimeter reading on successive days. I. Diabetic. II. Diabetic. III. H. L. W. (normal). (The curves are scaled to a common copper value.)

comparison. As in Fig. 2 one copper line only has been taken, the curves showing the polarimeter readings being scaled so that the ratio polarimeter reading to copper reducing value is unaltered. It will be seen that the diabetic curves approach the copper line from the opposite side to that in the normal case.

One of the "normal" cases (J. C. C. P.) presented features differing from those of the others. The subject had no diabetic history, and was perfectly healthy. Determination of his blood sugar by Bang's old method gave .17 p.c., which is abnormally high as is shown in the protocols. Even in the cases of feeding with sugar, this amount was not reached. The maximum amounts so obtained were .13 p.c. with G. F. T. after 150 grams of glucose, and .14 p.c. with W. S. after taking 100 grams of glucose. Further, the ratio of polarimeter to copper reduction value was higher than in other experiments on normal persons. The results we think were due to the psychological condition of the subject. He was somewhat nervous about the operation, which is not entirely painless and nervous influences can affect the sugar content of the blood

in a variety of ways. We determined his blood sugar next day by Bang's method, and obtained the figure  $\cdot 12$  p.c., which would tend to support this view.

One experiment was made on a case of cerebral hæmorrhage in which there was sugar in the urine. The subject was on a milk diet, was comatose and apparently unconscious that blood (100 c.c.) was being drawn. A large amount of sugar was present in the blood. The ratio polarimeter to copper reducing value was however much below that of the diabetic cases. The polarimeter value attained the copper value in 12 hours, the short time being no doubt due to the difference between them being originally slight. Thus the change was like that of normal, and not like that of diabetic subjects in which the polarimeter value approaches the copper line from the upper side.

### *Discussion.*

The nature of the normal blood sugar we leave for the present undecided. Since the sugar yields an osazone having the same melting point as glucosazone, and the same microscopic appearance, it is probably a hexose. The failure to obtain lævo-rotation may possibly have been due to a rapid change in the sugar notwithstanding the care taken to prevent it. Professor Hopkins suggested that if alcohol were used throughout as a precipitant, alteration of the sugar might be prevented. This was tried but the clear filtrate was dextro-rotatory.

The method takes much more time than that with tungstic acid. Alcohol allows fatty substances to pass into the filtrate, whilst by the tungstic acid method almost the whole of the fatty bodies remain entangled in the precipitate; and it was only after repeated filtering in the various stages that a clear fluid suitable for polarimetric observations was obtained.

On applying Seliwanoff's test for fructose, negative results were obtained, indicating the absence of lævulose and pseudo-lævulose (isoglycuronic acid).

The sugar we consider is some form of glucose. We have seen that the polarimetric readings finally reach a value corresponding to that given by  $\alpha$ ,  $\beta$  glucose in equilibrium. Further experiments on the final filtrate as soon as obtained showed that  $\text{KMnO}_4$  (12) was decolourised with great rapidity when compared with a solution of glucose of equal concentration; but after being allowed to stand for some days until the polarimeter reading has risen to a value corresponding to that of  $\alpha$ ,  $\beta$  glucose, the decolorisation is slow and approximates to that of  $\alpha$ ,  $\beta$  glucose. It is suggested therefore that the decolorisation by the fresh filtrate is due to the sugar and not to some impurity. It is in harmony with this view that, according to Hewitt and Pryde (9), sugar solutions



introduced into the intestine undergo a rapid downward muta-rotation. The enzyme responsible for the conversion being apparently absent from the blood, and the reaction of the blood being alkaline, suggests that it is impossible for so reactive a sugar to exist free in the blood. The combination, if combination there is, must be of the nature of an unstable glucoside for it is well established that the sugar of the blood is readily diffusible.

Experiments after taking glucose and fructose indicate that these sugars are rapidly changed into the normal form present in blood. Nef(13) suggested that the reaction  $d$ -fructose  $\rightarrow$  glucose must take place with the intermediary formation of an enolic form. It is probable then that the normal blood sugar is  $\gamma$  glucose, and that it arises from the enolic form.

While the work of Hewitt and Pryde(9) may account for the formation of  $\gamma$  glucose owing to passage through the intestinal wall, some further explanation is necessary as regards the sugar which is formed from glycogen. The experiment with J. C. C. P. suggests that  $\gamma$  glucose is formed not directly by glycogen breakdown, but as the result of the interaction of some other mechanism. We may therefore picture the processes:

glycogen  $\rightarrow \alpha, \beta$  glucose  $\rightarrow \gamma$  glucose occurring.

For the first stage of this process the liver diastase is the active agent, for the second stage the work of Clark(2, 3) suggests that the pancreas is responsible.

We have confirmed Hewitt and Pryde's observations(9) that extracts of the mucous membrane of the intestine do not alter the specific rotation of glucose solutions *in vitro*, and we can only suggest that as the result of some stimulus the pancreas secretes an enzyme, or enzymes, which cause the change  $\alpha, \beta$  glucose  $\rightarrow \gamma$  glucose. Some experiments on which we are at present engaged tend to support this view.

The marked difference shown between the curves of polarimetric readings obtained from normal subjects and severe diabetic patients throws a new light on the etiology of the disease. It is probable that glucose can only be stored and utilised after passing through the  $\gamma$  form, and that the severity of diabetes depends on the degree to which the power to form this sugar is lost. In the severe cases which we have examined there was no  $\gamma$  glucose which we were able to detect, any small amount present being masked by the large amount of other sugar in the solution. This would be especially the case in the diabetic where the very high ratio polarimeter to copper value suggests that a certain

amount of sugar may be present of which the ratio is higher than that of  $\alpha$ ,  $\beta$  glucose. We were unable to test this by acid hydrolysis owing to the small amount of filtrate in the final product. The further fact that the polarimeter curve does not reach the original copper value tends to support this hypothesis. It seems feasible to conclude that the direct cause of diabetes is the lack or inactivation of the enzyme which causes the conversion  $\alpha$ ,  $\beta$  glucose  $\rightarrow$   $\gamma$  glucose.

### SUMMARY.

1. A method is described for obtaining the sugar of blood in a concentrated solution free from proteins.
2. By means of this it is shown that the sugar in normal blood of man, and of the ox, sheep, cat and rabbit, is an unstable form of glucose, with an initial low rotatory power. It is suggested that the sugar is  $\gamma$  glucose.
3. Glucose and fructose taken in large quantities *per os* cannot be detected as such in the blood, their conversion into normal blood sugar being very rapid.
4. Nervous influences alter the nature and quantity of the blood sugar.
5. The blood sugar of persons suffering from severe diabetes mellitus is of an abnormal nature. It appears to be the  $\alpha$ ,  $\beta$  form of glucose.
6. An enzyme is postulated whereby the  $\alpha$ ,  $\beta$  equilibrium form of glucose is converted into  $\gamma$  glucose. This enzyme is absent from the blood.
7. The absence or inactivation of this enzyme is suggested as the cause of diabetes.

We wish to express our grateful thanks to Mr J. J. Walsh, M.B., B.Ch., who has been ready at all times to perform the operations of drawing blood from the normal subjects.

We are much indebted to Drs J. F. Gaskell, G. Graham, E. Lloyd Jones and J. Aldren Wright, for obtaining for us blood from the pathological cases.

We wish to thank the following who kindly volunteered to allow samples of blood to be taken from them: Messrs G. M. Dean, J. B. S. Haldane, H. Mallinson, J. C. C. Poole, G. F. Taylor and H. L. Wilson.

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## PROTOCOLS.

ANIMALS. One experiment only is given of those made on each kind of animal.

	Initial polarimeter reading	Final reading	Copper reducing value
	R.	R.	R.
Rabbit, 50 c.c. blood	·02	·07	·08
Cat, 80 c.c. blood ...	·03	·08	·08
Sheep, 100 c.c. blood	·04	·08	·08
Ox, 100 c.c. blood ...	·03	·12	·12

## EXPERIMENTS ON MAN.

	Polarimetric readings on successive days	Copper reducing value	Bang
	R.	R.	%
H. L. W. Normal. 95 c.c. blood ...	·07, ·08, ·09, ·11, ·11	·11	—
H. L. W. 150 gm. fructose. 100 c.c. blood	·04, ·06, ·09, ·10, ·10	·10	·13
G. F. T. Normal. 85 c.c. blood ...	·03, ·06, ·05, ·10, ·09	·10	—
G. F. T. 150 gm. glucose. 55 c.c. blood ...	·04, ·06, ·08, ·08, ·08	·08	·13
H. M. Normal. 72 c.c. blood ...	·02, ·03, ·08, ·10, ·10	·12	·10
J. C. C. P. Normal. 100 c.c. blood ...	·20, ·19, ·20, ·18	·21	·17
J. B. S. H. Normal. 70 c.c. blood ...	·08, ·09, ·10, ·10, ·10	·10	·17
G. M. D. Normal. 70 c.c. blood ...	·02, ·05, ·08, ·08, ·08	·08	·08
W. S. 100 gm. glucose. 65 c.c. blood ...	·05, ·07, ·09, ·08, ·08	·08	·14
L. B. W. Normal. 100 c.c. blood ...	·07, ·11, ·13, ·14, ·14	·14	·09
L. B. W. 100 gm. glucose. 55 c.c. blood ...	·04, ·05, ·08, ·09, ·09	·09	·13
L. B. W. 150 gm. fructose. 100 c.c. blood	·06, ·08, ·10, ·13, ·14	·16	·12
Diabetic (Dr J. Aldren Wright's patient). 18 c.c. blood ...	·11, ·10, ·09, ·09	·09	—
Diabetic (Dr J. Aldren Wright's patient). ( 20 c.c. blood ...	·11, ·10, ·10, ·10	·10	—
Diabetic (Dr G. Graham's patient). 21 c.c. blood ...	·11, ·10, ·08, ·08	·08	—
Diabetic (Dr G. Graham's patient). 13 c.c. blood ...	·07, ·06, ·05, ·05	·03	—
G. P. (cerebral hæmorrhage. Dr Lloyd- Jones' patient). 100 c.c. blood ...	·21, ·26, ·24, ·25	·25	—

# THE INFLUENCE OF CARBON DIOXIDE ON THE INTERCHANGE OF IONS BETWEEN THE CORPUSCLES AND THE SERUM OF BLOOD.

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MODERN views regarding (a) the transport of carbon dioxide in the blood and (b) the maintenance of the neutrality of the blood, are based on Hamburger's hypothesis that the red cell is freely permeable to anions. The doctrine of the impermeability of the red cell to kations has been put forward by a large number of observers—Gürber(1), Höber(2), Koeppe(3), De Boer(4), Overton(5), Doisy and Eaton(6), and Mukai(7)—and, in fact, has met with general acceptance. According to Hamburger(8), however, the cell envelope is not impermeable to kations. He regards the sodium and potassium ions as existing in a state of dynamic equilibrium with one another, and able to interchange with one another under certain conditions. Thus, when the quantity of carbon dioxide in equilibrium with blood is increased from 10 p.c. to 20 p.c., potassium passes into the corpuscle and sodium out.

The first experimental observations on the effect of carbon dioxide on the blood were made by Zuntz in 1867. He found that the alkali of the serum was increased in amount when blood was submitted to high tensions of carbon dioxide. Zuntz assumed that this phenomenon was due to the carbon dioxide splitting sodium from a sodium protein compound. The liberated sodium combined with carbon dioxide and formed sodium carbonate. By further absorption of carbon dioxide sodium bicarbonate was produced, which, by a process of diffusion, became equal in amount on both sides of the cell.

The permeability of red cells to the chloride ion was demonstrated by Nasse(9) in 1874, and subsequently, Hamburger and his school showed the general permeability of the red cell to anions ( $\text{—Cl}$ ,  $\text{—PO}_4$ ,  $\text{—SO}_4$ , etc.). On Hamburger's hypothesis the Zuntz phenomenon, i.e. the increased quantity of alkali present in serum after the treatment of blood with carbon dioxide, is assumed to be due to the passage of anions—chloride mainly—from the serum into the cell and not to the passage of sodium from the cell into the serum.

Gürber's suggestion that carbonic acid reacts with the sodium chloride present in serum forming sodium bicarbonate and hydrochloric acid, of which the latter diffuses into the cell, is an expression of these observations. Such an action might take place to some extent if the active mass of carbonic acid were large compared to that of the sodium chloride. But the fact that considerable quantities of sodium chloride are present, not only in the serum but also in the red cells, and that these cells are freely permeable to carbon dioxide appears to form an effective barrier against the acceptance of Gürber's hypothesis.

Quantitative experiments by Van Slyke and Cullen(10) have shown that after treating blood with carbon dioxide at high tension, only about one half of the additional carbon dioxide carried by serum can be accounted for by the change in the chloride ion concentration. These observations accord with those of McLean, Murray and L. J. Henderson(11), who find that with varying tensions of carbon dioxide only two-thirds of the bicarbonate is accounted for by chloride. On the other hand Fridericia(12) and Mukai(7) both find that the increase in the carbon dioxide capacity of the serum is exactly equivalent to the chloride loss.

The observations on the distribution of chloride and bicarbonate ions between the red cells and the serum, and the impermeability of the red cells to kations, have been submitted to mathematical analysis by Warburg(13), and according to him may be explained on Donnan's theory of the distribution of permeating ions in two phase systems containing non-permeating ions.

In the following pages the effects of carbon dioxide on the distribution of electrolytes between the cells and serum of blood are recorded. The correlation of the ionic interchanges between the corpuscles and serum, and the variations in the carbon dioxide carrying powers of these fluids, do not support the hypothesis that the red cell is permeable to anions only. Kations move freely across the envelope of the red cell under the influence of carbon dioxide and take an important part in the mainten-

ance of the neutrality of the blood, and the transport of carbon dioxide. Many of the experiments were carried out on blood saturated with carbon dioxide. It has been stated by Overton that results obtained after saturating the blood with carbon dioxide do not represent the changes which take place in the animal when the tensions of carbon dioxide vary between small limits only. This objection would be valid if (a) the red cell functions as a living cell, since high concentrations of carbon dioxide have a pronounced narcotic action on living tissues or (b) carbon dioxide decomposes substances in the red cell which are involved in preserving the neutrality of the blood. In point of fact the results observed on varying the carbon tension from 0 mm. to 40 mm. Hg, and from 0 mm. Hg to saturation vary in degree only. Further, the ionic interchanges which occur between the serum and corpuscles at high carbon dioxide tensions are completely reversible when the tension is lowered. Therefore the two objections are untenable and the results obtained on saturating blood with carbon dioxide may be accepted as showing in a gross degree the changes which occur with small variations in carbon dioxide tensions.

The experiments were made on fresh sheep's blood. It was impossible to carry out all the experiments on one sample of blood. Therefore, in order to correlate the results it was necessary to carry out the analyses on a series of different bloods and to average the figures. Each analysis is the result of two determinations which did not differ by more than 1 p.c.

*The relative volumes of red cells and serum in blood.*

Some of the results recorded depend upon the relative volumes of cells and serum in blood. To determine these values, 50 c.c. of the blood under examination was submitted to prolonged spinning in a powerful centrifuge (about  $\frac{1}{2}$  hour at 8000 to 10,000 revolutions per minute). At the end of this time the serum was carefully pipetted off, and measured. This method raises the question whether the degree of centrifugal force affects the relative volumes of cells and serum; that is, whether the fluid can be expressed from the red cells by centrifugal force. With any sample of blood a constant value can be obtained by prolonged spinning, beyond which no further separation can be made. This might be due to an equilibrium set up between the centrifugal force and the altered osmotic pressure of the red cell. To settle this question the volume of the red cells contained in a definite quantity of blood was determined. The red cells were then suspended in a volume of Ringer solution equal to that of the original blood and again centrifuged. The corpuscular

volumes in each case were identical. The specific gravities of the serum and the Ringer solution differed widely—1032 and 1010 respectively—and therefore the centrifugal force to which the corpuscles were submitted differed to a corresponding degree. Hence it appears that on spinning blood the corpuscles are not compressed and that the values obtained by this method give the true corpuscular volume.

Many of the experiments are recorded under the terms: "Normal blood," "Carbon dioxide free blood," and "Carbon dioxide saturated blood." "Normal blood" implies blood which has been put into equilibrium with the alveolar air of the lungs. The term " $\text{CO}_2$  free blood" is applied to blood through which  $\text{CO}_2$  free air has been aspirated for a considerable period of time (about 2 hours) until no further evolution of  $\text{CO}_2$  can be detected by baryta water. Such blood is never free from  $\text{CO}_2$  when tested with sulphuric acid. " $\text{CO}_2$  saturated blood" designates blood which has been submitted to the action of carbon dioxide until no more gas is absorbed.

The variation in the corpuscular volume of blood with varying carbon dioxide contents was described by Limbeck(14) in 1894, and can be readily observed by direct experiment. The degree of change, however, is a definite function of the time. This fact is evident from the following results which show the volume of serum obtained from 100 c.c. of normal blood, and from the same blood, saturated with  $\text{CO}_2$ , after varying times.

Blood (100 c.c.)	Time after saturation	Serum	Corpuscles
Normal	—	60 c.c.	40 c.c.
$\text{CO}_2$ saturated	5 mins.	52 "	48 "
"	2 hours	56 "	43.4 "
"	4 "	58 "	41.6 "

In the experiments recorded a period of approximately two hours elapsed in the preparation of the different samples of blood, so that this time factor may be regarded as constant. The following figures give the corpuscular volume for four different samples of blood, and the average values are those used in the calculations given in later experiments.

Volume of serum from 100 c.c. of blood:

	$\text{CO}_2$ free	Normal	$\text{CO}_2$ saturated
(1)	67 c.c.	66 c.c.	60.5 c.c.
(2)	63 "	63.5 "	60 "
(3)	60 "	60 "	55 "
(4)	60 "	60 "	56.6 "
Average	62.5 "	62.4 "	58 "
Average volume of corpuscles (by difference)	37.5 "	37.6 "	42 "

It may be assumed that under these experimental conditions the volume of the serum from  $\text{CO}_2$  free blood is identical with that from normal blood. Presumably during the process of abstraction of  $\text{CO}_2$  from the normal blood any alteration in volume resulting from the removal of the  $\text{CO}_2$  adjusts itself during the prolonged period necessary to remove the last trace of  $\text{CO}_2$ . Probably the  $\text{CO}_2$ , to which the red cell is freely permeable, increases the number of ions in the cell by the decomposition of some previously slightly ionised complex. The increased volume of the cell is caused by the rapid passage of water from the serum into the cell owing to the increased osmotic pressure and the subsequent diminution in volume is due to the slow diffusion of the increased ion into the serum until equilibrium is attained.

*The total alkali and potassium of the ash of blood and serum.*

*Method for total alkali.* It is a matter of considerable difficulty to obtain concordant results for the alkali of the ash of serum and blood. Apparently the salts of the alkalies are, to some degree, volatile at high temperatures. In view of the importance of the matter we put forward our method in detail. 20 c.c. of blood or serum were dried at  $100^\circ \text{C}$ . and then decomposed as completely as possible in an oven at  $300^\circ \text{C}$ . The carbonaceous residue was crushed as completely as possible with an agate pestle, and the platinum basin was then heated uniformly to very dull redness to decompose the residual organic matter. This operation was repeated and the ultimate residue was again very finely powdered. A small quantity of water was now added to the basin and gently warmed, and the contents were washed as completely as possible into a flask. The residue in the basin was ignited to oxidise the residual carbon, and 20 c.c. of 0.1 *N* hydrochloric acid added, the contents being again washed into the flask. In this way all the organic substances were decomposed, and all the inorganic matter was transferred to the flask without loss by volatilisation. The contents of the flask were now heated to about  $100^\circ \text{C}$ . in order that the salts in the interstices of any carbon particles might react with, or dissolve in the hydrochloric acid solution. When cool the whole of the contents were transferred to a 250 c.c. measuring flask, made up to the required volume with water, and left to stand for a few minutes. It was then filtered and 200 c.c. of the filtrate titrated against 0.1 *N* sodium hydroxide, using 6-8 drops of phenol phthalein as indicator.

*The total alkali of the ash of blood and serum.* The following results show the amounts of alkali present in the ash of (1) 100 c.c. of blood



and (2) 100 c.c. of serum from the blood. The figures express the power of the ash to react with acids (such as hydrochloric acid) in terms of c.c. of normal alkali. The final result is the average of four different samples of blood.

Alkali in terms of *N* NaOH in 100 c.c. of:

	Blood	Serum
(1)	3.75 c.c.	3.78 c.c.
(2)	3.53 "	3.47 "
(3)	4.5 "	3.94 "
(4)	5.19 "	4.0 "
Average	4.24 "	3.8 "

We may express the result by saying that the ash of blood contains alkali equal to 4.24 c.c. *N* NaOH and of this the serum contains 2.39 c.c. *N* NaOH, and the corpuscles 1.85 c.c. *N* NaOH. The amount of alkali in the ash of 100 c.c. of serum (3.8 c.c. *N* NaOH) is less than that in 100 c.c. of corpuscles (4.9 c.c. *N* NaOH), although the actual quantity of alkali in the ash of serum obtained from a definite volume of blood is greater than in the ash of the corpuscles contained in the same volume of blood.

The alkali of the ash of serum obtained from (a) normal blood, (b)  $\text{CO}_2$  free blood and (c)  $\text{CO}_2$  saturated blood. The ash of serum from blood containing varying quantities of  $\text{CO}_2$  contains variable quantities of alkali. The following figures give the quantities of alkali in the ash of serum obtained from four samples of blood, each sample of blood being divided into three parts and rendered (1)  $\text{CO}_2$  free, (2) normal and (3)  $\text{CO}_2$  saturated before the separation of the serum from it.

Alkali in terms of *N* NaOH contained in the ash of 100 c.c. of serum from:

	$\text{CO}_2$ free blood	Normal blood	$\text{CO}_2$ saturated blood
(1)	3.65 c.c. <i>N</i> NaOH	4.37 c.c. <i>N</i> NaOH	5.97 c.c. <i>N</i> NaOH
(2)	3.19 "	4.15 "	5.22 "
(3)	3.12 "	3.94 "	5.87 "
(4)	3.37 "	4.0 "	5.94 "
Average	3.33 "	4.125 "	5.75 "

When blood passes from  $\text{CO}_2$  free to normal there is a 23 p.c. increase on the alkali which may be obtained from the ash of the serum; and similarly from  $\text{CO}_2$  free to  $\text{CO}_2$  saturated a 72 p.c. increase in the alkali of the ash of the serum.

The potassium of blood and serum. Sodium and potassium form the predominant alkali ions of blood and serum. By determining the quantity of potassium present under any condition a fair approximation to the sodium value may be obtained by difference.

The potassium estimations were made on the neutralised filtrates

obtained after determining the total alkali contained in the ash of serum from any sample of blood.

These neutralised filtrates were evaporated to dryness on a water-bath. The residue was redissolved in water and filtered into a platinum dish containing 6-7 c.c. of perchloric acid solution. The remaining processes were those used in the ordinary estimations of potassium as perchlorate. The analyses show that potassium is present in small quantities only (0.116 p.c. as KCl) in sheep's blood and is approximately equally distributed between the serum and corpuscles.

	% potassium in blood	% potassium in serum
(1)	0.0579	0.0595
(2)	0.0653	0.0601
(3)	0.0650	0.0600
(4)	0.0642	0.0634
Average	0.0608	0.0622

The approximate equality in the amount of potassium in blood and serum suggests that the red cell is freely permeable to the potassium ion. There is certainly no indication from the analyses that the potassium ion preponderates in the corpuscle and the sodium ion in the serum, as is stated to occur in human blood by Wonach. If the potassium in the ash of serum be considered to exist as alkali and be calculated in terms of normal alkali, it is found that of the total alkali in the ash of serum 39 p.c. is due to potassium.

*The influence of carbon dioxide on the distribution of potassium between corpuscles and serum.* This effect is very much less than that observed with the total alkali contained in the ash of serum. The figures under A. show that the ash of serum obtained from normal blood contains a little less potassium than the ash of serum obtained from the same blood saturated with CO<sub>2</sub>. The change is about 6 p.c.; in a corresponding experiment with the total alkali of the ash the change was 39 p.c.

% potassium from 100 c.c. of serum from blood:

A.			B.		
	Normal	Saturated with CO <sub>2</sub>		Normal	Freed from CO <sub>2</sub>
(1)	0.0604	0.0637	(1)	0.83	0.80
(2)	0.0634	0.0674	(2)	0.66	0.64
(3)	0.0720	0.0766			
Average	0.0653	0.0690	Average	0.74	0.72

Similarly, when blood is freed from carbon dioxide a little potassium enters the corpuscle (cf. B.). In this case the change was about 3 p.c.; in a corresponding experiment with the total alkali of the ash the change

was 23 p.c. The small effect of  $\text{CO}_2$  on the distribution of potassium between the corpuscles and serum of blood, compared with the great effect of this gas on the total alkali of the ash suggests that sodium and not potassium enters into a specific relation with the red cell in the processes concerned in the transport of  $\text{CO}_2$  in the blood. Probably the potassium exists in the blood as an inorganic salt only.

*The chloride, phosphate and sulphate of blood and serum.*

It is generally assumed that the varying quantities of alkali in the ash of serum obtained from blood containing varying quantities of  $\text{CO}_2$ , are due to the transference of chloride from the red corpuscle to the serum, or *vice versa*, and not to any migration of the sodium or potassium ion. The previous figures, however, show that there is an actual change (that is, migration) of the potassium ion and suggest that there may be a similar definite change in the sodium ion. Moreover, calculations based on the total alkali of ash of serum under varying conditions and of the chief anions ( $-\text{Cl}$ ,  $-\text{PO}_4$ ,  $-\text{SO}_4$ ) under these conditions, show that there is a considerable transference of the sodium ion from the cell to the serum, or *vice versa*, when the  $\text{CO}_2$  of the blood is increased or diminished.

*Method of estimating chloride.* 20 c.c. of 20 p.c. metaphosphoric acid were added to 20 c.c. of blood or serum diluted with water. The mixture was made up to 250 c.c., and filtered after allowing it to stand for  $\frac{1}{2}$  hour. 5 c.c. of concentrated nitric acid and 25 c.c. of 0.1 *N* silver nitrate were added to 150 c.c. of the filtrate, the volume being made up to 250 c.c. and left overnight. 200 c.c. of the filtrate, to which 20 c.c. of ferric indicator had been added, were finally titrated against 0.05 *N* ammonium thiocyanate.

*Chloride in blood and serum.* An analysis of blood and serum shows, not only that much more chloride is contained in the serum than the corpuscles, but that the percentage in the serum is much greater than in the corpuscle. It was found that 100 c.c. of blood contained .347 g. of chlorine as chloride. Of this, the serum contained .247 g., and the cells .100 g. In terms of percentages, the serum contained .396 p.c., and the corpuscles .267 p.c. of chlorine. The percentage of chlorine in whole blood, calculated as sodium chloride was low—only .57 p.c.—although the tonicity of the blood, measured by hæmolysis in varying strengths of sodium chloride, did not differ from that of normal blood. It is of interest to observe, however, that normal blood does not hæmolyse in diminishing strengths of sodium chloride solution until the percentage has fallen to approximately .6 p.c.

*Chloride in the sera from (1) normal blood, (2) CO<sub>2</sub> free blood, (3) CO<sub>2</sub> saturated blood.* The transference of chloride from the serum to the corpuscles under the influence of CO<sub>2</sub> is a statement upon which all investigators are agreed. But this transference constitutes only a small portion of the total ionic interchange between the corpuscles and serum under the influence of CO<sub>2</sub>. The following figures give the percentages of chlorine as chloride in various sera obtained from two samples of blood.

% chlorine in serum from blood:

	CO <sub>2</sub> free	Normal	CO <sub>2</sub> saturated
(1)	.418 g.	.494 g.	.367 g.
(2)	.372 "	.367 "	.318 "
Average	.394 "	.385 "	.342 "

From these figures it is evident that when CO<sub>2</sub> free blood is put into equilibrium with alveolar air the percentage of chlorine in the serum diminishes .009 p.e.; and similarly, when CO<sub>2</sub> free blood is saturated with CO<sub>2</sub> the amount of chlorine in the serum diminishes by .052 p.e. Expressed in terms of percentages of the total quantity of chlorine present in the serum of CO<sub>2</sub> free blood, the loss from the serum to normal blood is 2.3 p.e.; and from the serum on passing to CO<sub>2</sub> saturated blood is 13 p.e. It may be observed that the variations are very much less than the variations met with in the alkali of the ash of sera obtained from corresponding types of blood.

*The total phosphate in the sera from (1) normal blood, (2) CO<sub>2</sub> free blood, (3) CO<sub>2</sub> saturated blood.* It appeared reasonable to assume that the phosphate anion might share in the migration process from the serum to the corpuscle under the influence of CO<sub>2</sub> in a manner similar to that observed with the chloride ion. A number of determinations were made by Neumann's method of the total phosphate contained in the different sera. The results were obtained with three different samples of blood.

Phosphate in terms of P<sub>2</sub>O<sub>5</sub> contained in 100 c.c. of serum from blood:

	CO <sub>2</sub> free	Normal	CO <sub>2</sub> saturated
(1)	.0259 g.	.0248 g.	.0242 g.
(2)	.0273 "	.0274 "	.0281 "
(3)	.0309 "	.0310 "	.0308 "
Average	.0280 "	.0277 "	.0277 "

It may be observed that there is a remarkable constancy in the quantity of total phosphate in the various sera in contrast to the chlorine results in similar experiments. The results indicate that the phosphate ion does not migrate when the quantity of CO<sub>2</sub> in the blood is varied. The small variations which occur are probably due to variations in the

degree of hæmolysis in the sera, since it is impossible to free blood from  $\text{CO}_2$ , or to saturate blood with  $\text{CO}_2$ , without rupturing some red cells. This hypothesis is supported by the fact that there is much more total phosphate (Neumann's method) in the corpuscles than in the serum of blood, as the following figures show.  $\text{P}_2\text{O}_5$  in blood .0476 p.c.; in serum .0284 p.c.; in corpuscles .0755 p.c.

*The inorganic phosphate in blood.* The whole of the phosphorus, whether present in organic combination or as an inorganic salt, is estimated as phosphate by Neumann's method. The above phosphate figures, therefore, may not be analogous to the chloride figures. A method was consequently adopted to estimate the inorganic phosphate present in blood and serum.

50 c.c. of blood or serum was diluted to 250 c.c. with 10 p.c. trichloroacetic acid solution in a standard flask. The resultant mixture was filtered and 150 c.c. of the filtrate was treated with an excess of magnesium citrate mixture and ammonia. The precipitate thus obtained was too small for accurate manipulation, indicating that there is practically no inorganic phosphate in serum or blood. This deduction offers a simple explanation why the phosphate ion does not migrate from the serum to the corpuscle in a manner analogous to that observed with the chloride radicle under the influence of  $\text{CO}_2$ .

*The sulphate of blood and serum.* The only other important inorganic acid radicle which might possibly play a part in the ionic migration under the influence of  $\text{CO}_2$  is the sulphate ion. Although the migration of this ion from serum to corpuscle has been described by De Boer<sup>(4)</sup>, the quantity of inorganic sulphate present in sheep's blood or serum is too small for accurate estimation by the usual methods. If the whole of the sulphate present in serum migrated to the corpuscles under the influence of  $\text{CO}_2$ , the total error caused by the omission of this factor would be negligible.

*The reversibility of the change in alkali and chloride in serum under the influence of carbon dioxide.*

The reversibility of the migration of alkali and chloride from the serum to the corpuscles, or *vice versa*, under the influence of  $\text{CO}_2$  was investigated. The following figures give the quantity of alkali in terms of *N* NaOH contained in 100 c.c. of serum obtained from (1) blood freed from  $\text{CO}_2$ , (2) blood saturated with  $\text{CO}_2$ , (3) blood first saturated with  $\text{CO}_2$ , and then freed from it. Similar results were given by the chloride estimations from the same sera.

Alkali in terms of  $N$  NaOH contained in the ash of 100 c.c. of serum from:

(1) 3.65 c.c.; (2) 5.07 c.c.; (3) 3.37 c.c.

Chloride in 100 c.c. of serum from:

(1) .416 g.; (2) .367 g.; (3) .409 g.

It is clear from the above figures that the migrations of alkali and chloride from the serum to the cell, under the influence of  $CO_2$ , are completely reversible. The result appears to dissociate the possible vital activity of the red cell from the processes involved in the transport of  $CO_2$ , since saturation with  $CO_2$  has a very toxic effect on all living matter.

*The average quantities of carbon dioxide combined with blood and serum.*

*Normal blood and serum.* The following figures give the percentage amounts of combined  $CO_2$  in a series of bloods and corresponding sera, when put into equilibrium with alveolar air.

Percentage of carbon dioxide in:

	Blood	Serum
(1)	56.2	42.0
(2)	50.5	57.2
(3)	52.7	47.2
(4)	54.5	55.0
(5)	58.0	46.0
(6)	63.0	58.0
Average	55.8	50.0

*The carbon dioxide capacities of sera obtained from (1) normal blood, (2)  $CO_2$  free blood, and (3)  $CO_2$  saturated blood, when put into equilibrium with alveolar air.* In the previous pages the ionic interchanges which take place between the corpuscle and the serum when the quantity of  $CO_2$  in blood is altered, have been detailed. The sera obtained from these different kinds of blood possess very different capacities for carrying  $CO_2$ . This fact is illustrated in the following series of figures obtained from different samples of blood. All the sera were put into equilibrium with alveolar air before their  $CO_2$  contents were determined. The figures give the combined  $CO_2$  only.

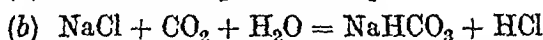
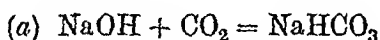
Percentage of combined  $CO_2$  in alveolated serum from blood:

	$CO_2$ free	Normal	$CO_2$ saturated
(1)	27.5	55	77
(2)	25	46	81
(3)	36	58	88
(4)	29.4	48.3	70.5
Average	29.5	51.8	84.1

## DISCUSSION OF RESULTS.

Certain general conclusions may be drawn regarding the effects produced by carbon dioxide on the blood from the average figures given in the previous pages.

The inorganic kations and anions of blood are mainly sodium, potassium, chloride, phosphate and sulphate. From the analyses it is clear that only the sodium and the chloride ions enter to any extent in the ionic interchange. Therefore, all the effects observed are worked out in terms of these ions and the correlations are made by using the two equations:



according to which 1 c.c. *N* NaOH = 22.24 c.c.  $\text{CO}_2$  = .0355 g. Cl.

*The alkali of the ash of various sera compared with the corresponding capacities of the sera to carry carbon dioxide.*

	(1) $\text{CO}_2$ capacity (alveolated)	(2) Variation in $\text{CO}_2$ capacity	(3) Total alkali of ash as <i>N</i> NaOH	(4) $\text{CO}_2$ equivalent of NaOH	(5) Variation in $\text{CO}_2$ cap. of ash
100 c.c. serum from					
Normal blood	51.8 %	} 22.3 %	4.125 c.c.	92 c.c.	} 16.7 c.c.
$\text{CO}_2$ free blood	29.5 "		3.33 "	75.3 "	
$\text{CO}_2$ saturated blood	84.1 "		5.75 "	128.0 "	
$\text{CO}_2$ saturated blood (corr. for vol.)	78.0 "		5.33 "	119.0 "	

It is evident from these figures that the carbon dioxide equivalent of the ash (col. 4) is much greater than the carbon dioxide carrying power of the corresponding serum when put into equilibrium with alveolar air (col. 1). But the change in the carbon dioxide equivalent of the ash of the different sera (col. 5) is approximately equal to the change in the carbon dioxide carrying powers of these sera (col. 2) when the quantity of this gas in the blood from which they have been obtained is varied. This constant relation indicates that in the serum the carbon dioxide is carried as sodium bicarbonate.

*The difference between the carbon dioxide equivalent of the ash and the capacity of the corresponding serum to carry carbon dioxide.*

100 c.c. serum from	$\text{CO}_2$ capacity (alveolated)	$\text{CO}_2$ equivalent of ash	Difference
Normal blood	51.8 c.c.	92.0 c.c.	40.2 c.c.
$\text{CO}_2$ free blood	29.5 "	75.3 "	45.8 "
$\text{CO}_2$ saturated blood	84.1 "	128.0 "	43.9 "
$\text{CO}_2$ saturated blood (corr. for vol.)	78.0 "	118.5 "	40.5 "

It may be seen that the  $\text{CO}_2$  equivalent of the ash always exceeds the  $\text{CO}_2$  capacity of the corresponding serum, although these  $\text{CO}_2$  capacities vary so widely as 29.5 p.c. and 78.0 p.c. This difference is represented by some organic sodium salt in the serum. The quantity of sodium thus combined is considerable (approximately equal to that combined with  $\text{CO}_2$  in the serum of normal blood). Possibly this sodium is combined with the protein of the serum and constitutes a second reserve of alkali (in addition to that supplied by the red blood corpuscles) which may be drawn upon when the  $\text{CO}_2$  of blood is increased in quantity. This suggestion is supported by the fact that the excess of alkali in the ash (last column) progressively diminishes as we pass from  $\text{CO}_2$  free blood to normal blood, and from normal blood to  $\text{CO}_2$  saturated blood. Serum proteins, therefore, act not only as a source of alkali reserve but also as a means of preserving the neutrality of the blood.

*The change in the chloride concentration compared with the change in the carbon dioxide capacity of the corresponding serum*

100 c.c. serum from	% of Cl	Change in Cl conc.	$\text{CO}_2$ equiv of Cl change	$\text{CO}_2$ capacity of serum	Difference
Normal blood	382	} 012 g 052 " 076 "	7.5 c.c.	51.8 c.c.	} 22.3 c.c. 29.5 " 84.1 " 78.0 "
$\text{CO}_2$ free blood	304		32.5 "	29.5 "	
$\text{CO}_2$ saturated blood	342		49.0 "	84.1 "	
$\text{CO}_2$ saturated blood (corr. for volume)	318			78.0 "	

It is evident from these figures that the passage of chloride from the serum to the corpuscles under the influence of carbon dioxide accounts for only one third of the increased capacity of the serum to carry this gas on passing from  $\text{CO}_2$  free blood to normal blood. On saturating blood with carbon dioxide a much greater quantity of chlorine enters the corpuscles. Under these circumstances the change in the concentration of the chloride ion accounts for the whole of the increased carbon dioxide capacity of the serum obtained from  $\text{CO}_2$  saturated blood.

*The effect of an altered distribution of alkali between the corpuscles and serum on the capacity of the blood to carry carbon dioxide.* The sera obtained from  $\text{CO}_2$  free blood and  $\text{CO}_2$  saturated blood possess widely different capacities to carry  $\text{CO}_2$  when put into equilibrium with alveolar air. This variation in  $\text{CO}_2$  capacity has been shown to be accompanied by similar variations in the alkali content of the corresponding sera and corpuscles. Thus, in  $\text{CO}_2$  free blood there is a large quantity of alkali in the ash of the corpuscles and a small quantity in the ash of the serum, whilst in  $\text{CO}_2$  saturated blood there is a small amount of alkali in the ash of the cor



puscles and a large amount in the ash of the serum. It is of interest to observe that these large differences are not associated with any change in the total carbon dioxide carrying powers of the two constituents of each type of blood. Thus, the following figures were obtained:

100 c.c. of  $\text{CO}_2$  free blood were separated into 62.4 c.c. of serum and 37.6 c.c. of corpuscles. The serum put into equilibrium with alveolar air combined with 43 p.c. of  $\text{CO}_2$ . Therefore, 62.4 c.c. of serum contained 26.8 c.c. of  $\text{CO}_2$ . The corpuscles suspended in .85 p.c. NaCl combined with 45 c.c. of  $\text{CO}_2$ . Therefore, the total  $\text{CO}_2$  held in combination by the corpuscles and serum of the original blood was 71.8 c.c. of  $\text{CO}_2$ .

100 c.c. of the same blood was saturated with  $\text{CO}_2$  and then separated into 58 c.c. of serum and 42 c.c. of corpuscles. The serum, put into equilibrium with alveolar air, combined with 86 p.c. of  $\text{CO}_2$ . Therefore, 58 c.c. of serum contained 50 c.c. of  $\text{CO}_2$ .

The corpuscles suspended in .85 p.c. NaCl combined with 21 c.c. of  $\text{CO}_2$ . Therefore, the total  $\text{CO}_2$  held in combination by the corpuscles and serum of the original blood was 71 p.c. of  $\text{CO}_2$ .

The equality of these results shows that it is a matter of indifference whether the available alkali is held in the serum or corpuscles as far as the capacity to carry  $\text{CO}_2$  is concerned when put into equilibrium with alveolar air. It may, therefore, be assumed that if the  $\text{CO}_2$  is present in the serum as sodium bicarbonate a similar fact holds true for the  $\text{CO}_2$  in the corpuscle. This deduction implies that the hæmoglobin of the red cell does not combine with carbon dioxide.

#### SUMMARY.

(1) The increased volume of the corpuscles of blood with increased carbon dioxide content is a definite function of the time. This indicates that carbon dioxide increases the number of ions in the cell by the decomposition of some slightly ionised compound, and that the liberated ion slowly diffuses across the envelope of the red cell.

(2) There is a 23 p.c. increase in the alkali of the ash of serum on passing from  $\text{CO}_2$  free blood to normal blood; and a 72 p.c. increase in the alkali of the ash of the serum on passing from  $\text{CO}_2$  free blood to  $\text{CO}_2$  saturated blood.

(3) Potassium is contained in small quantities only in sheep's blood (.116 p.c. as KCl). Under the influence of carbon dioxide the transference of potassium to the cell or from the cell is small (about 5 p.c.). Probably, potassium exists in blood as an inorganic salt only, and does not enter into any specific relation with the contents of the red cell.

(4) The serum loses 2.3 p.c. of its total chloride (expressed as chlorine) when blood passes from  $\text{CO}_2$  free to normal blood; and 13 p.c. of its total chloride on passing from  $\text{CO}_2$  free to  $\text{CO}_2$  saturated blood.

(5) There is only a trace of inorganic phosphate in sheep's blood. No appreciable change takes place in the phosphate content of serum under the influence of  $\text{CO}_2$ .

(6) The quantity of inorganic sulphate in sheep's serum is too small for accurate estimation by the ordinary methods.

(7) There is a large difference between the alkali of the ash and the capacity of the corresponding serum to carry  $\text{CO}_2$ . This represents an organic sodium compound in the serum—possibly protein—and constitutes a source of reserve alkali when the quantity of carbon dioxide in the blood is increased.

(8) The differences in the alkali of the ash of different sera prepared from the same blood is approximately equal to the differences in the carbon dioxide carrying powers of these sera. This constant relation indicates that carbon dioxide is carried as sodium bicarbonate in the serum.

(9) The change in the chloride concentration of the serum accounts for one-third of the change in the carbon dioxide carrying power of the serum on passing from  $\text{CO}_2$  free blood to normal blood. Hence, sodium must pass freely from the red cell to the serum when the tension of carbon dioxide to which the blood is subjected varies from 0 to 5 p.c. of an atmosphere. The whole of the change in the carbon dioxide carrying power is, however, accounted for by the change in the chloride concentration on passing from  $\text{CO}_2$  free blood to  $\text{CO}_2$  saturated blood.

(10) The total quantity of carbon dioxide carried by the cells and serum of  $\text{CO}_2$  free blood is the same as that by the cells and serum of  $\text{CO}_2$  saturated blood, although the distribution of alkali between the cells and serum attains a maximum difference in these two kinds of blood. This shows that carbon dioxide is carried by the cells in the same way as in the serum—as sodium bicarbonate—and that the hæmoglobin of the red cell does not combine with carbon dioxide.

(11) The large effects produced by carbon dioxide on the distribution of sodium and chlorine between the serum and corpuscles compared with the small effect produced by it on the distribution of potassium, indicates that sodium and chlorine enter into a specific chemical relation with some constituent of the red cell, which is not shared by the other ions of the blood.

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## PITUITARY SECRETION. BY W. E. DIXON.

(From the Pharmacological Laboratory, Cambridge.)

THIS communication deals with the conditions which cause secretion of the pituitary gland to meet the needs of the animal economy. Cushing and Goetsch(1) examined the cerebro-spinal fluid of patients for the presence of "pituirrin": they used the physiological method and claimed to obtain effects corresponding with those produced by the posterior lobe of the pituitary. Carlson and Martin(2) failed to obtain a pressor effect by injecting the cerebro-spinal fluid of normal dogs into other animals; Cow confirmed Cushing, and Herring(3) found no evidence of pituirrin in the cerebro-spinal fluid of thyroid-fed or thyroidectomised cats.

In the present experiments dogs only were used: they were anaesthetised first with ether and then by injections of morphine and urethane. Tracheotomy was performed. The blood-pressure was taken from the right femoral artery and all injections were made into the right femoral vein. The cerebro-spinal fluid was collected from the sub-cerebellar cisterna by the method used by Dixon and Halliburton(4) and samples were collected for analysis at fixed intervals generally of fifteen minutes. It was essential in some experiments to obtain all the cerebro-spinal fluid available immediately after an injection, and in these instances a slight negative pressure was used.

*Normal cerebro-spinal fluid.* Samples of normal cerebro-spinal fluid of the dog show every known chemical and physiological action of pituirrin. The active substance is insoluble in absolute alcohol and in ether but is soluble in water. It is destroyed by digestion with trypsin but not with pepsin. It contracts the uterus and blood vessels, raises blood-pressure, increases the urinary flow and under suitable conditions causes a secretion of milk.

The amount of pituirrin in normal cerebro-spinal fluid varies greatly, and it is necessary to adopt some standard for comparison. After several trials I used the pituirrin of Parke Davis & Co., in sealed ampoules, different samples of which vary only slightly in activity. It was found that ten drops of normal cerebro-spinal fluid contain from 1 to 10 mgms. of this "pituirrin."

The quickest and most convenient method for determining the

quantity of pituitrin in cerebro-spinal fluid is by comparing the action of the fluid on the isolated uterus of the virgin guinea-pig with that of the standard pituitrin. One horn is attached to a suitably weighted lever and immersed in a bath of Ringer's fluid (Locke's modification) at 39° C. The bath contained 80 c.c. of fluid which could be readily replaced by fresh Ringer in 5 or 6 seconds. Each uterus requires to be standardised first by determining the minimal amount of standard pituitrin which will evoke a maximal contraction. This is essential since a sensitive uterus may enter into maximal contraction when the amount of pituitrin in the bath does not exceed 1 in 100,000, whilst occasionally a uterus is found which requires 1 in 5000 to produce a like effect. Cerebro-spinal fluid may be added in drops to the bath directly, since it is practically a Ringer's solution, and thus a comparative effect is easily obtained. After each dose of either pituitrin or cerebro-spinal fluid the Ringer must be changed and a sufficient time given, generally about 10 minutes, for complete relaxation. In this way a number of samples can be tested on the same horn. Occasionally I have seen as little as 10 drops of normal cerebro-spinal fluid produce a decided contraction of the uterus but the usual amount required to elicit an effect is 3 or 4 c.c., and occasionally even larger amounts are inactive. This is not remarkable since it is reasonable to suppose that pituitrin is secreted in variable amounts to meet the needs of the body.

*Experimental.* The present communication is characterised by the very large number of negative experiments. No form of nerve stimulation, sensory nerves, vagus and sympathetic in the neck, caused any change in the secretion of the pituitary. The inhalation of oxygen and carbon dioxide is likewise without effect. Certain drugs were injected intravenously or given by inhalation but all with negative results. Thus alcohol and chloroform were given by inhalation because they increase the flow of cerebro-spinal fluid; urea and caffeine because of their diuretic or cerebral stimulant action; histamine, 20 mgms., because of its marked action on the uterus; adrenalin, and  $\beta$  telluronium dichloride because the latter specifically excites the suprarenal glands; several alkaloids and glucose were inactive. One typical experiment will explain the procedure.

PROTOCOL 1. Dog, male, 20 kilos cerebro-spinal fluid collected as follows:

A. Normal fluid.

At 11.50, 3 c.c. 1 %  $\text{Te}(\text{CH}_3)_2\text{Cl}_2$  given.

B. Fluid collected from 11.50 to 11.55

C. " " " 12.20 " 12.30

D. " " " 12.50 " 12.60

E. " " " 2 " 2.10

These samples were then tested on the uterus.

5 drops	pituitrin (1 in 100)	=maximum contraction.
5	" "	" "
50	" cerebro-spinal fluid	A=nil.
70	" "	B="
50	" "	C="
70	" "	D="
80	" "	E="
2	" pituitrin (1 in 100)	=maximal contraction.
200	" cerebro-spinal fluid	A=nil.
1 drop	pituitrin	=maximal contraction.

(The normal cerebro-spinal fluid in this dog contained hardly any pituitrin.)

Each of the other drugs mentioned were tried on at least two occasions and gave negative results.

Preparations of the posterior lobe of the pituitary injected into the general circulation cause pituitrin to appear immediately in the cerebro-spinal fluid. It is present in excess in about a minute after the injection and the cerebro-spinal fluid is again free from such excess in five minutes. This effect can be repeated an indefinite number of times.

Protocol 2. Dog, male. Cerebro-spinal fluid collected as follows:

1. Normal.
2. Immediately after intravenous injection of choroid extract.
3. 20 minutes " " " " "
4. 60 " " " " "
5. Immediately " " " " 2 c.c. pituitrin.
6. 20 minutes " " " " 2 " "

The cerebro-spinal fluid was tested for pituitary on the uterus.

No effect produced by samples 1, 2, 3, 4 and 6 in 30-drop doses.

3 drops of 5 = slight contraction.

6 " " 5 = maximal "

(This corresponded with pituitrin in cerebro-spinal fluid, 1 in 100.)

Certain crystalline drugs are excreted into the cerebro-spinal fluid but always in such minute amounts that they are difficult to detect even by the most delicate chemical reagents: traces of salicylates and urotropine are so excreted. Halliburton and I showed that the current flows in the opposite direction, foreign crystalloids injected into the cerebro-spinal fluid pass into the general circulation with ease and great rapidity. The facts do not suggest that the presence of pituitrin in the cerebro-spinal fluid under the conditions named, is through the circulation. First because the amount of pituitrin is so large: if an injection of 10 c.c. 1 in 4 pituitrin is made into a vein, the concentration of pituitrin in the cerebro-spinal fluid reaches a strength of from 1 in 100 to 1 in 500. Moreover other uterine stimulants such as histamine, which has a relatively small molecule, when injected in 20 or 30 mgm. doses is not excreted by the cerebro-spinal fluid in amounts detectable by the physiological method. For these reasons I conclude that the secretion of

pituitrin under these circumstances is specific. That is, either the pituitary gland picks up the excess of pituitrin from the blood just as the

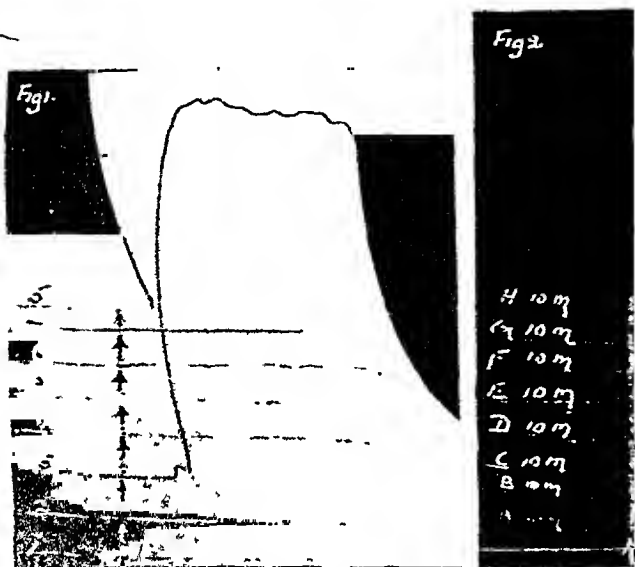


Fig. 1. For details see Protocol 2.

Fig. 2

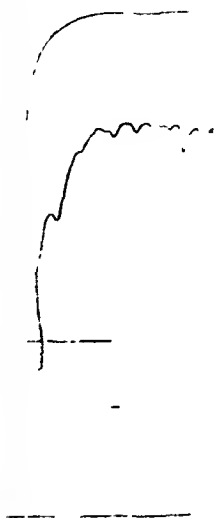


Fig. 2. For details see Protocol 5.

liver picks up bile salts, the process in each case causing secretion—or conceivably pituitrin acts as a pure stimulant to the gland.

The view that pituitrin might reach the cerebro-spinal fluid by the circulation was made improbable by three experiments, one on a dog and two on cats in which the pituitary gland was destroyed by the cautery from the ventral surface. Pituitrin was injected into the circulation before and after destruction and it was found that injections after destruction did not alter the amount of pituitrin in the cerebro-spinal fluid. The following protocol is typical.

Protocol 3. Dog, male, 16 kilos. Normal cerebro-spinal fluid (A). 2 c.c. pituitrin in 10 c.c. Ringer's solution injected and the fluid collected during the next ten minutes (B). The spinal cannula was then removed and the pituitary destroyed by the cautery. (This was confirmed in the post-mortem examination.) After an interval of half-an-hour the spinal cannula was reinserted and a sample of cerebro-spinal fluid collected (C). A second injection of pituitrin identical with the first was given and the fluid collected in the next ten minutes (D).

The blood-pressure of the dog remained high throughout the experiment.

A, C and D had no action on the uterus in 20-drop doses though in 30-drop doses each caused a similar though sub-maximal contraction. B caused maximal contraction in 15-drop doses.

Pituitrin therefore stimulates the pituitary gland just as bile salts stimulate the liver. This experiment shows that the cerebro-spinal fluid

after extirpation of the pituitary still contains some pituitrin, and I have no explanation to offer of this phenomenon. What purpose the pituitrin serves in the cerebro-spinal fluid is not clear, but any excess rapidly disappears. Halliburton and I (5) showed that when it is introduced into the cerebro-spinal fluid it rapidly reaches the circulation and exerts its ordinary specific effects on plain muscle throughout the body. A balance appears to be struck between the amount in the blood and in the cerebro-spinal fluid. An excess in the blood is absorbed by the gland and some of it reaches the cerebro-spinal fluid: an excess in the cerebro-spinal fluid is excreted rapidly into the blood.

*Animal extracts.* Extracts of different tissues were prepared by pounding the fresh organ with sand adding about double the weight of saline, boiling and filtering. The extracts were injected slowly into the femoral vein and the cerebro-spinal fluid examined at intervals for excess of pituitrin. The following extracts gave negative results: liver, brain, choroid extract of brain, testis, epididymis, anterior lobe of pituitary, thyroid and pancreas. One protocol of these experiments is given which exemplifies all.

PROTOCOL 4. Dog, male. Cerebro-spinal fluid collected as follows.

1. Normal cerebro-spinal fluid.
2. Immediately after injection 2 c.c. pituitrin.
3. 15 minutes " " 2 " " "
4. 15-45 " " " " liver extract of rabbit.
5. 45-60 " " " " " " "
6. 1, 2 hours " " " " " " "
7. Immediately after a further injection 2 c.c. pituitrin.

Numbers 2 and 7 caused maximal contraction of the uterus in 15-drop doses. The others were inactive in 20-drop doses.

Ovarian extract is the only animal extract apart from pituitary so far examined which produces an immediate secretion of the pituitary gland. The earlier experiments were made from fresh tissue. After such an injection pituitrin appears in the cerebro-spinal fluid in a minute or two and the action is over in ten minutes. The fluid takes a little time to fill and drop from the cannula which may appear to increase the latent period; to avoid this as far as possible a little gentle suction was used.

PROTOCOL 5. Dog, female. One ovary removed between ligatures and a saline extract made. Cerebro-spinal fluid collected as follows.

- A. Normal fluid.
- B. Cerebro-spinal fluid collected during asphyxia.
- C. " " " 30 minutes after.
- D. " " " up to 5 minutes after ovarian extract.
- E. " " " 15 " "
- F. " " " immediately after pilocarpine. "
- G. " " " 2 c.c. pituitrin.
- H. " " " up to 5 minutes " "



All the samples were inactive except D and H, which caused maximal contraction in 10-drop doses. This effect corresponds with a normal cerebro-spinal fluid, 1 c.c. of which contains 1 drop of pituitrin.

In a further series of experiments the sterile extracts of Parke Davis were used. These were of three kinds. (1) The whole ovary. (2) The ovary from which the corpus luteum had been removed. (3) Corpus luteum. The corpus luteum was without any action on the pituitary secretion but the other two extracts immediately excited the pituitary to secrete: the secretion of pituitrin commenced about 20 seconds after injection and was present in excess for a minute or two when the cerebro-spinal fluid became normal. The effect was repeated on the same animal several times. The record of one experiment is shown in Fig. 3. The increase of tone with pituitrin is abrupt; with the cerebro-spinal fluid it is more gradual. Perhaps the secretion in the cerebro-spinal fluid is not quite in the same form as that of "pituitrin." Iscovesco(6), Aschner(7), Hermann(8) and others have shown that extracts of corpus luteum induce hyperæmia and hyperplasia of the muscle and mucous membrane of the uterus. Observations have also been recorded suggesting that pituitary extracts exert a like action, but more extended observations have disproved this view.

The facts seem clear that ovarian conditions determine the secretion of the pituitary and thus react indirectly on the uterine tonus. Pituitrin is very largely employed in medicine to contract the uterus: its employment would seem to be so far rational that it is the drug manufactured by the body for this specific purpose.

*The effect of alimentary extracts.* Cow(9) working in this laboratory on diuresis believed that water taken by the mouth was a better diuretic than water injected subcutaneously, because in its passage through the alimentary canal it absorbed some substance which excited the pituitary. The secretion of the pituitary is influenced by injecting boiled and filtered extracts of mucous membrane but the effect is of a different nature from that caused by ovarian injections. With ovarian extract the latent period is in seconds and the effect is rapidly over. With the intestinal mucous membrane the effect is delayed and the augmented secretion may not be noted for an hour; the percentage increase of pituitrin in the cerebro-spinal fluid is not so great as after ovarian extract but it remains in the fluid longer. I repeated Cow's observations on seven animals and obtained a positive result in five though one of these was not very decided. After such a long latent period the flow of fluid may be small and find a more ready absorption by the vessels than by outflow through the cannula and this may account for the failures.

The effect is not protein shock since practically no protein is present in the injections. Nor does the long latent period suggest that it is a

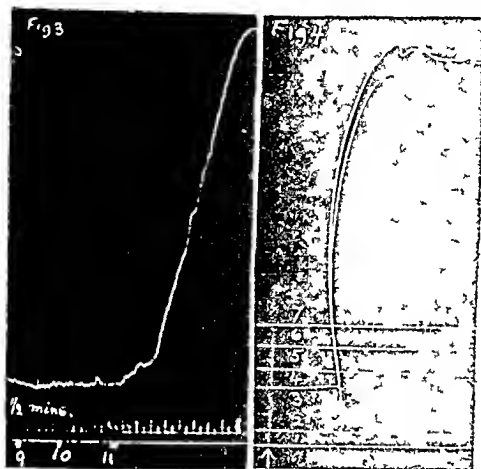


Fig 3 Record of isolated uterus. Fig 4 For details see Protocol 6

At 9 24 drops normal cerebro spinal fluid added

At 10 15 drops cerebro spinal fluid ten minutes after injection of ovarian extract without corpus luteum

At 11 15 drops cerebro spinal fluid 1 minute after injection of ovarian extract without corpus luteum

direct effect on the pituitary. To test this point I injected the extract into the central end of the carotid artery in two further experiments, and these gave completely negative results within the next hour. However this effect is produced we only know the beginning and end points. The following protocol is typical.

**PROTOCOL 6** Dog, male Duodenum removed between ligatures and the mucous membrane extracted in saline, boiled and filtered. Cerebro spinal fluid collected as follows

7	10 drops normal cerebro spinal fluid
6	10 "
5	10 " " " " 15 minutes after injection
4	10 " " " " 35 " "
3	5 " " " " 60-120 " " Diges 37° C 18 hours
2	10 " " " " 60-120 " " "
1	10 " " " " 60-120 " " " 37° C 18 "

1 drop of trypsin to 25 drops cerebro-spinal fluid incubated for 18 hours at 37° C. Controls without trypsin.

2 and 3 produced maximal contraction of the uterus. No other sample produced any effect.

The experiments appear to me conclusive that some constituent of the intestinal mucous membrane so affects metabolism that about one hour or more after its liberation, the pituitary is induced to secrete.

If the pituitary is a remnant of a gland once secreting into the alimentary canal, as Gaskell believed, it is perhaps not extraordinary that its secretion should be associated in some way with the functions of the alimentary canal. The prolonged latent period which injections of mucous membrane require before the pituitary secretes makes it certain that the pituitary takes no active part in the immediate processes of digestion. I was therefore led to investigate its action on absorption. Definite quantities of tap water were placed in 6-in. loops of intestine chosen from various parts of the alimentary canal in anæsthetised cats and dogs. Every care was taken to ensure an efficient circulation. The normal slow absorption which occurs was entirely uninfluenced by injections of pituitary. The experiments were repeated with the same results on surviving intestines.

During these observations another phenomenon was observed. Pituitary extract exerts a very decided effect in increasing the tone of the small intestine without effect on peristalsis. This effect is, of course, well recognised and the modern treatment of post-operative atony of the gut is by pituitary injections. This action is peculiar to the small intestine. The large intestine behaves differently: the first effect of injection is to increase the tone for from 10 to 20 seconds; this is followed by a marked relaxation of tone at a time when the effect is maximal in the small intestine. This effect is shown graphically in Fig. 5, which is a record of two experiments on two cats. A loop of six inches of intestine was tied at one end and fitted with a cannula at the other. This cannula communicated with a Mariotte's bottle and the records in the lower curve represent bubbles of air replaced from the upper part of the bottle as more fluid passes into the intestine. The water or saline was at a pressure of 10 cms. The upper tracing is from the small intestine, the normal rate of absorption can be gauged from the fact that seven bubbles correspond with 1 c.c. The effect of injection of pituitary is to diminish the number of bubbles, that is to increase the tone since the rate of absorption remains constant. The lower tracing (B) is from the large intestine; the first effect is to increase the tone to such an extent that the water rises in the tube. The effect is followed in a little over a minute by profound

relaxation, an effect which I have never observed in the small intestine. This phenomenon may be the result of the direct action of the pituitary

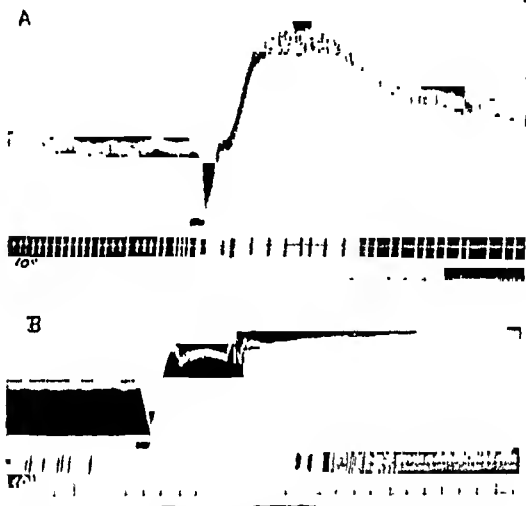


Fig. 5. Record of blood-pressure in two cats, the middle curve in each experiment is described in text, A in the case of the small intestine and B the large intestine.

on muscle. Such a highly specific effect seems inherently improbable but it cannot be dismissed off-hand since we know that on isolated and surviving arteries the effect of pituitrin may be different according to the situation of the excised portion. On the other hand it may be a reflex phenomenon, that conditions which throw the small intestine into tone may relax the large intestine: this is telologically understandable.

The cardiac sphincter is relaxed as food moves towards it in the oesophagus: the duodenum is inhibited during peristalsis of the pyloric end of the stomach (10) and Cannon (11) states that "as food is nearing the ileo-colic valve the large intestine is usually quiet and relaxed." The passage of food through the ileo-colic valve causes relaxation of the colon which occurs in the absence of nervous connections with the spinal cord (12). The pituitary reaction is I think different from these: the intestine is isolated and the tube for recording was always inserted at

least an inch from the ileo-colic sphincter. The result is receiving further consideration.

The pituitary exerts another function on digestive processes. By some means it regulates the amount of sugar in the blood: it depresses hyperglycæmia whether produced by anæsthetics or adrenaline, and the evidence points to the fact that it bears some relation to glycogenesis in the liver. It has been suggested that one cause of the diabetic condition may be pituitary insufficiency. This is, also, a property of the posterior lobe and I have mentioned that extracts of the anterior lobe, anæsthetics, adrenaline, and excess of sugar in the blood do not influence the secretion of pituitrin.

#### CONCLUSIONS.

1. The pituitary gland secretes into the cerebro-spinal fluid.
2. Pituitary extract injected into the circulation causes the gland to secrete. Pituitary extract injected into the cerebro-spinal fluid rapidly causes the ordinary systemic effects by passing into the general circulation. A balance is struck between the amount in the blood and cerebro-spinal fluid.
3. Ovarian extract specifically excites the gland to secrete. The effect is immediate and lasts only two or three minutes. The active substance is not in the corpus luteum.
4. Duodenal extract causes a secretion after one hour, but neither so much nor so constant as that caused by ovarian extract, the effect however is more prolonged. Some suggestions as to its significance are offered.
5. Pituitary extract increases tone in the small intestine, and diminishes tone in the large intestine.

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## VARIATIONS IN THE CO<sub>2</sub> CONTENT OF THE BLOOD CONSTITUENTS IN RELATION TO MEALS<sup>1</sup>.

BY E. C. DODDS AND J. McINTOSH.

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It has been shown (1) that a meal causes first a rise, and then a fall of alveolar CO<sub>2</sub> tension. And reasons have been given (2,3) for considering that the rise is due to the secretion of acid by the gastric glands, and the fall to the secretion of alkali (bicarbonate) by the pancreatic and intestinal glands.

In the present investigation the CO<sub>2</sub> content of the whole blood, of the plasma and of the corpuscles in their relation to the alveolar CO<sub>2</sub> have been determined.

### *Methods.*

Owing to the extreme difficulty of obtaining volunteers who will suffer themselves to be bled more than twice during an investigation, specimens of blood were collected before a meal, at the height of the gastric secretion, or at the height of pancreatic secretion. The subjects, again all healthy men whose gastric response had previously been investigated by a continuous test-meal, were rested in the laboratory for half an hour before the experiments were begun. Their alveolar CO<sub>2</sub> tension level was determined at this stage by the Haldane-Priestley method(4). When constant, blood was collected from the median basilic vein under aseptic precautions, using a syringe and needle sterilised in paraffin at 130°C., no tourniquet, of course, was used; amounts varying from 7 to 10 c.c. were taken, and were expelled into a centrifugal tube, the sides of which had been dusted with a finely powdered neutral potassium oxalate. The tube was filled with blood, and the point of the needle was left in the tube, and on forcing in the rubber stopper all air was expelled through the needle, which was then removed. Two tubes of blood were collected at each vein puncture. One of the tubes was at once centrifuged, whilst the other blood was examined as detailed below. The above technique was practised, and, with trained assistants, the blood was undergoing the appropriate

<sup>1</sup> A Report to the Medical Research Council.

examination in a very short time after its removal from the subject. Speed is absolutely essential if a near approximation to the condition of the blood in the body is required. All the examinations described below were performed on each specimen of blood obtained.

*Whole Blood.* The  $\text{CO}_2$  contents of the whole blood was determined before and after meals by the method recently described by Haldane<sup>(5)</sup>. This was found to give good results. In the Table will be seen the result of such experiments.

TABLE.

CO <sub>2</sub> in plasma (Van Slyke's method)		pH of plasma (McClendon's method)		TABLE. CO <sub>2</sub> in vols. % (Haldane's Method)								Alv. tens.	
Before After		Before After		Whole blood		Plasma		Corpuscles		Before			
Before After		Before After		Before	After	Before	After	Before	After				
taken during maximum rise													
64.2	64.2	{ a 7.80 b 7.40 }	{ 7.82 7.40 }	58.5	67.0	27.5	27.0	30.0	39.1	40.2			
64.2	64.2	{ a 8.08 b 7.40 }	{ 8.13 7.50 }	57.0	66.5	28.5	28.5	27.5	37.0	42.3			
68.7	68.7	{ a 8.16 b 7.40 }	{ 8.20 7.42 }	54.5	63.0	35.6	35.7	19.3	27.0	41.9			
65.4	65.4	{ a ? b 7.49 }	{ ? 7.49 }	53.5	59.0	26.7	27.0	26.4	31.0	41.0			
71.9	72.0	{ a 8.25 b 7.48 }	{ 8.30 7.55 }	54.0	61.5	26.5	27.0	28.5	34.0	35.2			
72.1	72.9	{ a 7.90 b 7.42 }	{ 7.90 7.64 }	49.0	59.0	25.0	25.0	26.0	34.0	37.3			
68.3	68.5	{ a 7.80 b 7.40 }	{ 7.90 7.50 }	48.1	56.0	24.1	24.0	23.9	31.0	35.2			
control													
62.1	62.1	{ a ? b 7.40 }	{ ? 7.40 }	48.2	48.6	25.5	25.6	23.12	22.9	36.0	3		
68.4	68.4	{ a 7.90 b 7.40 }	{ 7.95 7.40 }	49.0	49.5	24.0	24.1	24.3	24.9	36.1	36		
during maximum fall													
61.1	61.1	{ a 7.9 b 7.45 }	{ 7.9 7.5 }	47.2	40.0	20.0	20.0	27.27	21.2	40.0			
63.0	63.0	{ a 7.8 b 7.4 }	{ 7.8 7.4 }	42.6	35.9	17.5	17.6	25.9	18.4	43.2			
61.2	61.2	{ a 7.85 b 7.4 }	{ 7.8 7.4 }	51.0	40.5	20.9	20.4	31.0	20.3	44.7			
ers 1 to 7 show the effect of													

Numbers 1 to 7 show the effect of gastric secretion upon the blood. The second specimen was taken when the alveolar  $\text{CO}_2$  tension had reached its highest point after the meal. Numbers 7 and 8 are starvation and achlorhydric controls respectively. Numbers 10, 11 and 12 show the effect of pancreatic secretion upon the blood. The second specimen was obtained when the alveolar  $\text{CO}_2$  tension had reached its lowest point after the meal. In the column giving the pH (a) refers to the solutions obtained by adding an equal volume of water to plasma and exhausting, (b) when N/50 acid was used in place of the distilled water.

It can be seen that the CO<sub>2</sub> content of the blood rises and falls *pari passu* with the tension of the alveolar CO<sub>2</sub>.

*Plasma.* The plasma from the centrifugalised blood was examined before and after meals for its CO<sub>2</sub> capacity. In the Table a very striking series of results are seen. The CO<sub>2</sub> content of the plasma appears to remain practically constant throughout the digestive changes.

*Corpuscles.* After the plasma had been pipetted off the blood, the nozzle of a clean pipette was plunged into the tightly packed corpuscles, and a known quantity was taken up and examined for CO<sub>2</sub> content. The results were checked by calculation, taking into consideration the CO<sub>2</sub> content of the whole blood, plasma, and the hæmatocrit reading. The calculated and experimental values were found to agree so closely that only the experimental values were recorded. No attempt was made to wash the corpuscles, as any interference or undue manipulation would have upset the CO<sub>2</sub> content. In the Table it is seen that all the blood CO<sub>2</sub> changes occur in the corpuscles, and that the plasma takes practically no part in them whatsoever. The CO<sub>2</sub> content of the corpuscles varies directly with the alveolar CO<sub>2</sub> tension, being high at the height of gastric secretion, and low at the height of pancreatic secretion, as determined by the maximum elevation or depression of the alveolar CO<sub>2</sub> tension. Further evidence on the absence of change in the plasma was obtained by investigating the alkali reserve by Van Slyke's (6) method, and by McClendon's (7) method. In the former the amount of NaHCO<sub>3</sub> present is calculated from the volume of CO<sub>2</sub> obtained by adding acid to a definite volume of plasma, and then evacuating it. In the latter method a definite volume of *N*/50 acid is added to plasma, the mixture is evacuated, and its hydrogen-ion concentration is measured by means of the hydrogen electrode. To one part of the plasma, an equal volume of distilled water was added whilst to a second an equal volume of *N*/50 HCl was added. These results, together with the Van Slyke readings, are found in the Table. It can be seen that the changes before and after meals are negligible.

#### SUMMARY.

1. The CO<sub>2</sub> content of the whole blood rises during gastric secretion and the concomitant elevation of alveolar CO<sub>2</sub> tension.
2. The CO<sub>2</sub> content of the whole blood falls during the period of depression of the alveolar CO<sub>2</sub> tension which has been associated by previous experiments, with the pancreatic secretion.



3. The plasma shows practically no change during the period of gastro-intestinal secretion, either as regards  $\text{CO}_2$  content or alkali reserve.

4. All changes in  $\text{CO}_2$  content following meals occur in the corpuscles.

The expense of this research to one of the investigators (E. C. D.) was borne by the Medical Research Council.

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# THE EFFECT OF LACTATION ON OVULATION.

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of Medicine for Women.)*

THE effect of lactation on the cyclic changes in the ovary of the rat and of the guinea-pig was investigated by Loeb and Kuramitsu<sup>(1)</sup> in the course of a study of the involution of the uterus in these animals. They came to the conclusion that in the rat ovulation is suspended during lactation, while in the guinea-pig it continues to take place. They state that ovulation in the rat occurs on an average every four or five days: This result is in agreement with that of Long and Evans<sup>(2)</sup>, who, however, point out that the length of the œstrous cycle is very variable, being often as long as 10 or 12 days. Ovulation, they agree, is suspended during pregnancy; it occurs spontaneously as a rule within the first 24 hours following labour, and is then suspended during the period of lactation. If lactation is greatly prolonged, i.e. beyond the 21 days after which the young can be weaned, ovulation may occur spontaneously before the end of nursing.

On this basis there could be no second litter in the interval between the time taken for gestation + 1 day (24 days), and this time + the period of lactation, i.e. until 45 days after the birth of the first litter. In some previous experiments<sup>(11)</sup> on the effect of thymus feeding, it was found that the second litter were born at various intervals after the first, and not in two sets corresponding one to the end of the first gestation, and the other to the end of the lactating period. In other words, the experiments gave no indication that ovulation ceased during lactation.

It was desirable in the first place to repeat the experiments in rats fed in the normal way. This we have done, and we have obtained similar results. The following table gives examples:

Rat	1st litter	2nd litter	Interval of days
1	May 26 (10)	June 29 (9)	34
2	April 21 (9)	May 22 (8)	31
3	Aug. 22 (10)	Sept. 24 (9)	31
4	Feb. 11 (7)	March 12 (7)	29
5	Aug. 26 (9)	Sept. 25 (10)	28
6	Oct 3 (9)	Oct. 30 (8)	27
2	May 22 (8)	June 17 (9)	26
3	July 30 (3)	Aug. 22 (10)	23

Similar cases have been recorded by Miss H. King(3), in the case of rats, and by J. F. Daniell(4) and W. B. Kirkham(5, 6), in the case of mice. These results can hardly be explained by variation in the period of gestation. In the very great majority of cases the duration of gestation varies by two or three days only. In a former experiment(11) one of us found the usual gestation period to be 23 days: Long and Evans in a recent investigation(12) found it to be  $21\frac{1}{2}$  to 22 days. Greater variations do occasionally occur, but they are too infrequent to account for the results mentioned above.

Kirkham(5, 6) explains the results as due to delayed implantation in the case of mice that are suckling young, the full activity of the mammary gland being the chief cause of delay. This explanation seems to us forced, in view of the fact that ovulation during lactation is recognised as occurring in other animals.

The experimental method adopted was to examine the ovaries towards the end of lactation to note the age of the corpora lutea. Long and Evans(7) inferred the absence of ovulation during lactation from results obtained by staining luteal cells with vital dyes. They found that if certain vital dyes were injected into the abdominal cavity just before parturition, the luteal cells already formed showed particles stained with the dye, and that if injected at any time during lactation one additional set of corpora lutea with cells stained by the dye were present, these being the corpora lutea resulting from the ovulation occurring within 24 hours of parturition. We were unable to obtain the dye used by Long and Evans: since, however, it is stated by Goldmann(8), by Evans and Schulemann(9), and by Monroe Sutter(10), that other vital dyes, such as trypan blue, are stored in the luteal cells, we carried out some injection experiments using trypan blue, trypan red, and Congo blue. The method of fixation in formalin, cutting frozen sections in gum, and counterstaining, was essentially that recommended by Goldmann in his original paper. Sections of the ovary gave good staining of various wandering cells in the connective tissue of the corpus luteum and in the ovarian stroma, and of cells of follicles undergoing granular degeneration, but never in the luteal cells themselves: in other organs, the storage of dye occurred as anticipated from the results of Goldmann and others. It is possible that the luteal cells take up the stain only at a certain stage of growth or degeneration. In a recent paper by Long and Evans(12) (which only came to our notice when writing our results), it is stated, however, that trypan blue stains luteal cells very faintly, and that in hardened specimens the preservation was unsatisfactory.

This method having proved inapplicable, the ovaries were examined with the object of ascertaining from the appearance and arrangement of the luteal cells the approximate age of the corpora lutea present. In all cases the mother was allowed to suckle her young, and was killed three weeks after the birth of the litter. The appearance of the ovaries was very constant, and in all young corpora lutea were found, but not in great numbers; this was probably associated with the lateness of the season. Some old corpora lutea were found, more or less well preserved, some vacuolar; these probably originated from the ovulation directly following labour. Some very young ones could be seen in which the cavities were as yet incompletely organised, and a certain amount of remnants of degenerated corpora lutea, the luteal cells having become broken down, and forming irregular clumps in the stroma of the ovary; these were the remains of the corpora of gestation. The follicles were comparatively few in number, and of varying size, some quite large, some well preserved, some in granulosa degeneration.

From the presence of young corpora lutea in these ovaries, it is concluded that ovulation does occur during lactation in the albino rat.

Long and Evans<sup>(12)</sup> have made use also of the method of the vaginal smear; they state that ovulation changes this, and that during lactation there is no such change. From this again they conclude that ovulation is suspended during lactation. We have not ourselves made trial of this method of investigation.

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## ON THE NATURE OF HISTAMINE ACTION.

By R. J. S. McDOWALL.

*(From the Department of Physiology, University of Leeds.)*

THE object of the experiments with which this investigation deals is to study in some detail the cause of the fall in systemic blood-pressure, which occurs when a small dose (0.1 mg.) of histamine is injected intravenously.

In 1918, Dale and Richards<sup>(1)</sup> showed most conclusively that the action of histamine is on the capillaries, and later Dale and Laidlaw showed that the condition of histamine shock was due to increased capacity of the vascular system and lessened output of the heart. The early part of the fall in systemic blood-pressure produced by large doses of histamine they considered to be accelerated by pulmonary constriction which greatly diminished the flow of blood to the left side of the heart. With regard to the fall of blood-pressure which follows small doses, Dale and Richards (1, p. 163) state that "it is not a volume effect due simply to increased capacity of the system, but to a diminished peripheral resistance." This conclusion they based on a careful and elaborate series of perfusion experiments in which they found that there was a diminished peripheral resistance if histamine was added to the perfusion fluid (Ringer-Locke solution containing blood corpuscles and a trace of adrenalin). This evidence was supported by the result found by Dale and Laidlaw<sup>(2)</sup> that histamine produced larger excursions of the lever in cardiometer tracings; this was interpreted as indicating an increased output of the heart. From these experiments therefore it was assumed that more blood-pressure reached the heart during the action of histamine, a result which would be expected if the peripheral resistance was diminished.

The immediate impulse which led to the commencement of the present investigation came from finding that the venous pressure did not always rise as would be expected if there was a diminution of peripheral resistance and if more blood passed through to the veins. Moreover, when it rose, neither its onset nor its magnitude bore any constant relation to the systemic change, as might with reason be expected if they were due to the same cause.

*Method.* All experiments were made on cats anæsthetised in the first instance with ether; when deeper nmæsthesia was required a ehloroform-ether mixture, or chloroform alone, was used. Venous pressure was recorded by n method described elsewhere(3).

The question of dose was first considered. If under light anæsthesia a small dose such as  $\cdot 01$  mg. was given, there was a rise of venous pressure; as the dose was increased the rise became less and when the shock dose was approached there was usually a fall in venous pressure. These results appeared to support the conclusions of Dale and Richards and the results of Connet(4) who obtained a fall of venous pressure with relatively large doses ( $\cdot 5$  mg.). But in view of the fact that Dale had called attention to the action of anæsthetic in making carnivora sensitive to the histamine, the depth of anæsthesia was varied. It was found that tho venous response was markedly altered. Under light anæsthesia with a dose of  $\cdot 01$  mg. there was a rise of venous pressure as stated above, but under deep and prolonged anæsthesia there was n venous fall, although the systemic fall was about the samo in both instances (Fig. 1). An explanation for this venous nltération was sought for. It might be said that the general sensitivity had increased and that the small dose was now having the effect of a large dose. This may readily be dismissed as the fall in arterial pressure does not necessarily change in magnitude although the venous response alters completely. An alteration in venous response without modification of the arterial suggests in itself that they are due to different causes.

It might also be said that deep and prolonged anæsthesia weakened the heart and that the histamine would then stimulate it, causing it to empty itself more effectively and so lower the venous pressure. While such anæsthesia is liable to cause high venous pressure through cardiac weakening, the same fall of venous pressure occurred in some cases when the venous pressure was not abnormally high (Fig. 2). Even if histamine stimulates the isolated heart, it does not follow that it stimulates the heart in the body. Dale and Laidlaw in their earlier paper came to the conclusion that the heart was weakened, and the occurrence of obvious cardiac failure on the injection of a small dose of histamine in an animal whose heart is already weak shows that the total effect of the histamine in the heart is to reduce rather than to improve cardiac efficiency. Further, when the pulmonary circulation is impaired or paralysed there is no evidence that the heart is benefited by histamine. Such benefit would show itself by a rise in pulmonary pressure, but at the stage just mentioned lessened efficiency of the heart may be evidenced

by irregularity of the heart's action and a fall in the pulmonary pressure (Fig. 2D). It is therefore concluded that cardiac effects following the

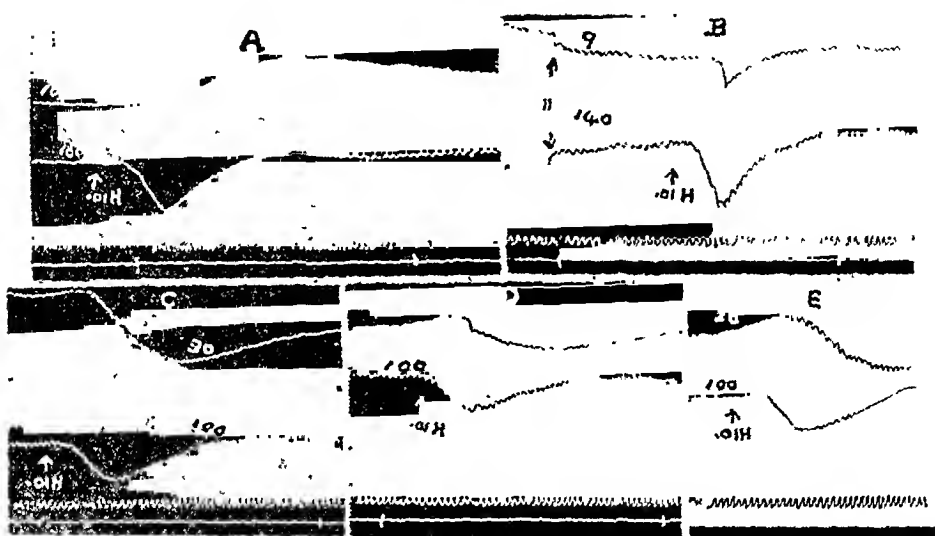


Fig. 1. Cat 3. 1 kg. c.E. mixture. Effect of varying anaesthesia on the change of venous blood-pressure caused by injecting 0.01 mg. histamine. The upper tracing is the venous pressure in mm. H<sub>2</sub>O; the lower tracing is the carotid pressure in mm. Hg. Natural respiration. Time in minutes. At each arrow 0.01 mg. of histamine was injected.

A. Ordinary sufficient anaesthesia. Rise of venous pressure.

B. Later, anaesthesia throughout, corneal reflex still present, only slight rise of venous pressure. The primary venous fall is due to an inspiratory gasp seen in respiration tracing.

C. Anaesthesia increased. Venous pressure large fall.

D. Anaesthesia lessened. Venous pressure fall decreased.

E. A few minutes later, rise of venous pressure.

injection of histamine are insufficient to explain the change in the venous pressure response under deep anaesthesia.

The most satisfactory explanation appears to be that the rise of venous pressure which occurs under light anaesthesia is one due to backward pressure or obstruction in the pulmonary circulation, and that the anaesthetic removes the pulmonary obstruction. Histamine as first shown by Dale and Laidlaw(2) using the method of Bradford and Dean(5), constricts the pulmonary vessels to a marked degree (cp. Fig. 2A). They have shown in relation to large doses that the sharp initial fall in systemic arterial pressure is due to this cause, and it will be seen below that even with small doses this factor may make itself manifest. When it is remembered that the right ventricle is

already impaired by the lowered and falling aortic and coronary pressures, it is not difficult to imagine that it is no longer able to empty itself against the increased resistance in the pulmonary vessels.

Now it has been shown by Brodie and Dixon that the action of the vagus on the bronchial muscles may be greatly reduced or may be obliterated by deep anaesthesia. I have found that the action of adrenaline on the bronchioles may be similarly reduced and have brought forward evidence<sup>(8)</sup> that the action of amyl nitrite on the pulmonary vessels may be prevented by deep chloroform anaesthesia. This is presumed to be due to the local action of the anaesthetic on the lungs themselves. When the effects of anaesthetics on living tissue generally are considered it is indeed difficult to see how the lung tissues can escape serious impairment when exposed to anaesthetic vapour for prolonged periods.

As has been said above, the rise of venous pressure following a small dose of histamine disappears under deep and prolonged anaesthesia, and I have suggested that the rise is due to backward pressure from the lungs. Demonstration that the pulmonary constriction is reduced or abolished by such anaesthesia would be strong circumstantial evidence in favour of the suggestion. Experiments were therefore made to test this point. Pulmonary pressure was recorded by the method of Sharpey Schafer<sup>(9)</sup> and it was found that on varying the depth of anaesthesia a series of changes parallel to those in the venous pressure could be obtained when histamine was injected. At first under light anaesthesia there was the typical rise in pulmonary pressure (Fig. 2A), but as the anaesthetic was deepened there was a delay in the pulmonary rise (this point was first noted by my colleague Mr Winfield who witnessed the experiment) and later it lessened (Fig. 2B) and then it disappeared entirely (Fig. 2C). If the experiment be prolonged there may be an actual fall of pulmonary pressure (Fig. 2D) which may be considered to be due partly to cardiac weakness and partly to less blood reaching the right side of the heart.

The results just given lead me to consider that the fall of systemic blood-pressure caused by a small dose of histamine in an animal under light anaesthesia is due mainly, if not entirely, to pulmonary constriction causing decreased output of the left ventricle and not to decreased capillary resistance. Thus the histamine effect does not indicate that variation in capillary resistance plays an important part in the circulation as Dale and Richards suggest it does. Any increased flow into the veins is probably annulled by increased capillary capacity. The



experiments of Dale and Richards mentioned above were made on tissues removed or isolated from the body and the results are not

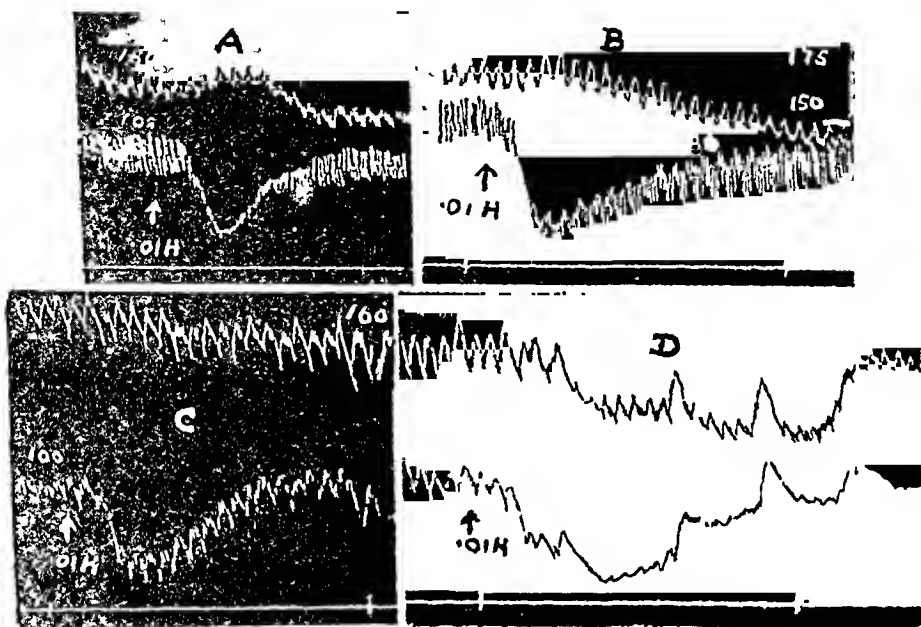


Fig. 2. Cat. c.e. mixture. Effect of varying anæsthesia on the blood-pressure in the pulmonary artery. Upper tracing, pulmonary blood-pressure in mm. H<sub>2</sub>O. Lower tracing, carotid pressure in mm. Hg. At each arrow 0.1 mg. of histamine injected. The anæsthetic was increased A to D.

A, rise of pulmonary artery blood-pressure. The rise is often much more marked.

B, a slight rise; C, no rise; D, a fall.

necessarily applicable to the normal circulation. It is true the evidence is deficient on two points. (1) That the rise of venous pressure never occurs unless there is pulmonary constriction and (2) that the pulmonary constriction is always sufficient to prevent the heart emptying itself normally. Owing to other factors which arise these points do not seem to be capable of absolute experimental proof.

The increased pulmonary resistance caused by histamine accounts for some of the other effects which it produces or may produce.

(a) It was noted by Bayliss in some unpublished experiments privately communicated to me that the venous rise caused by a small dose of histamine sometimes did not occur until the systemic blood-pressure was beginning to return to its normal level. This delay can, I find, be brought about by the anæsthetic and it is the natural result of the decreasing excitability of the pulmonary tissue to histamine.

(b) Dale and Laidlaw noted that on the injection of a large dose of histamine, the systemic fall frequently took place in two stages. The first stage they considered was due to pulmonary constriction cutting off the blood from the left side of the heart, the second to increased capacity of the system. In one experiment I obtained a similar fall in two stages on injecting a small dose of histamine (Fig. 3). If the first

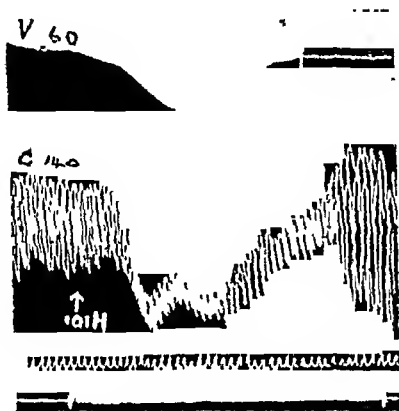


Fig. 3. Fall of systemic blood pressure caused by a  $\cdot 01$  mg. histamine. (This was the only case under light anaesthesia observed in which  $\cdot 01$  mg. caused a fall of venous pressure.) Two stages seen.

stage in one case is due to pulmonary constriction, it may be assumed to be so in the other. In this experiment there was a fall in venous pressure (Fig. 3) so that in this experiment the effect of the pulmonary constriction was more than counterbalanced by the increased capacity of the capillaries just as ordinarily occurs on injecting a large dose of histamine.

(e) I have also found that in shock when the capillaries are already dilated, there is still a rise in venous pressure. Such a rise could not be due to more blood passing through to the veins as there was evidence of increased peripheral resistance, indicated by a rise of the systemic pressure from arteriole constriction. Investigation of the pulmonary circulation at this stage showed that the pulmonary rise could still be obtained, although the usual systemic arterial fall did not occur. It is

assumed then that the venous rise was due to the pulmonary constriction.

### SUMMARY.

The parallelism between the disappearance under deep anæsthesia of the rise of pressure in the pulmonary artery and the rise of venous pressure which occurs as the result of the injection of a small dose of histamine suggests that they are both due to the same cause, namely, pulmonary constriction which is affected by the anæsthetic. Other results are given which support this view.

If the anæsthetic is increased so as to abolish the pulmonary effect, there is a fall of venous pressure although the systemic arterial fall may not have altered, nor the heart have been weakened.

The results indicate that the fall of arterial pressure which occurs on the injection of a small dose of histamine is the result of diminished output of the heart, due partly to pulmonary constriction, and partly to less blood reaching the heart as a result of increased capacity of the capillaries, and not, as Dale and Richards hold, to decreased capillary resistance.

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OBSERVATIONS ON THE RESPIRATORY CENTRES  
IN THE CAT. BY THOMAS LUMSDEN, M.D. (*Aberd.*).

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In the course of investigations on the working of the respiratory mechanism in cats, dogs, rabbits and monkeys, I have made observations on the effect on respiration of section of the brain stem at different levels. In this paper I give an account of the effect of such section in the cat. The observations of earlier observers have been made chiefly on the rabbit, and it will be seen that the results in the cat differ in some important points from those obtained by others in the rabbit.

*Method.* The cats were anaesthetised with ether, and a cannula placed in the right carotid for taking the blood pressure. The vagi were separated from surrounding tissues, and a thread placed under them, ready either to be cut or frozen. The parietal bone was trephined just in front of the occipital ridge, the left carotid being clamped during the operation, and if there was free bleeding, the vertebral arteries were compressed. A thin but rather blunt chisel was inserted just behind the tentorium, and pushed rapidly but gently downwards to the base of the skull, so that the brain stem was severed but the basilar artery remained intact; thus internal

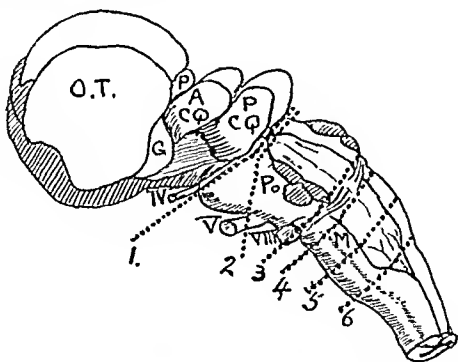


Fig 1. Diagram of brain stem showing level of crucial sections.

are then taken. Following these there is again a prolonged inspiration, and so on. The cycle repeats itself with great regularity for, it may be, two or three hours, but the duration of the inspirations gradually lessens.

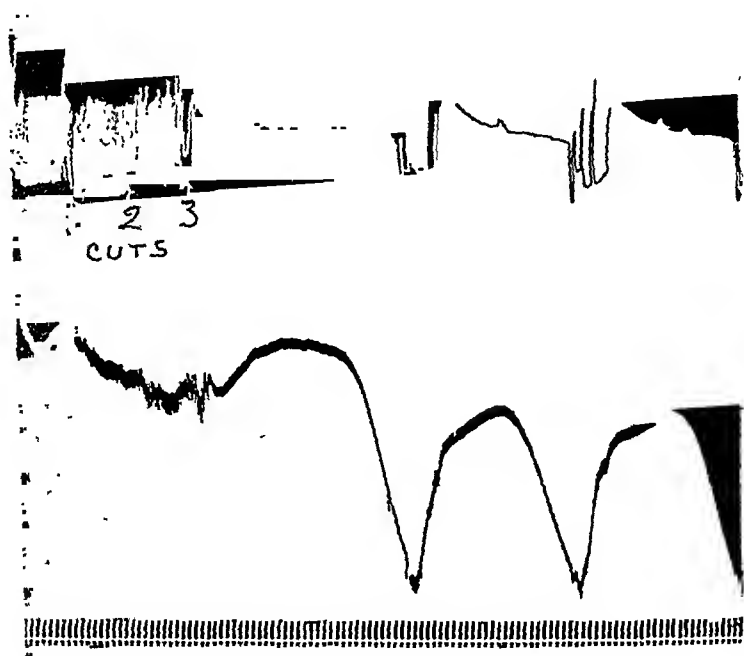


Fig. 3 Respiratory and blood-pressure tracings in a cat. Cut 1 was through the mid-brain; Cut 2 at sect. 1, Fig. 1; Cut 3 at sect. 2, Fig. 1. Insp. upwards. Abscissa 12 mm. below time tracing (5 secs.).

The blood-pressure tracing, taken simultaneously (Fig. 3), shows that during prolonged inspiration the arterial pressure first rises, and later sinks to a low level. It rises again rapidly during the short period of relatively quick respirations. The latter, then, are probably caused by the venous state of the blood, and the oxygenation they produce re-establishes the prolonged respiratory tonus, as if by reviving the central mechanism upon which it depends. The state is closely similar to that described by Marekwald and others in the rabbit, as following section below the posterior corpora quadrigemina when the vagi and fifth nerves are also cut. Trevan and Boock(6), in their recent account of experiments in the cat, describe the respiration as becoming very much deeper and slower on section of the vagi after severance of the brain stem just behind the posterior corpora quadrigemina, and cutting off about a third of the pons. My section 2 (Fig. 1) does not exactly correspond with this

cut, but from my experiments I should expect the section as figured by Trevan and Boeck to cause the prolonged inspirations without section of the vagi.

*Section through the level of the striæ acousticae* between lines 3 and 4 (Fig. 1) causes a prolonged incoordinate inspiratory spasm or convulsion of the diaphragm, and thereafter gasping respiration alone is seen, or, gasping interspersed with a few incoordinate inspiratory spasms. A similar result usually follows intracranial attempts to cut the seventh and eighth nerves, but this does not happen when these nerves are destroyed from the external auditory meatus, and the effect is due to hæmorrhages into the pons at this level dragging on the nerves.

*Section immediately below the striæ acousticae* (sect. 4, Fig. 1). Severance here also produces characteristic results, and they follow immediately on the section. Rhythmic respirations continue, but both inspiration and expiration are more sudden in beginning and ending. They are a series of brief gasps, such as those which occur between the prolonged inspirations after section at the upper part of the pons. The change is produced whether the vagi are intact, or frozen, or cut, so that it appears to be independent of the vagal impulses, though, of course, these are to some extent cut off by the section itself.

*Section between lines 5 and 6* causes the cessation of all respiratory movements and death.

The respiratory tracing of animals dying from asphyxia, anæsthetics, or experimental interference, very frequently shows, one after the other, each of the types of respiration described above (Fig. 4 *b*). From being regular, the breathing first becomes slow; then a series of prolonged inspirations and gasps appear and finally life is maintained for a short time by gasping alone. Conversely, when an animal which has been so nearly dead as to cease breathing, is revived by means of artificial respiration, the respiration returns in the reverse order, a preliminary period of gasping is followed by a series of prolonged inspirations which gradually shorten until they become indistinguishable from slow natural breathing, and soon the normal respiratory rhythm is resumed (Fig. 4 *a*).

#### *Remarks.*

It is clear from the previous account that if the descriptions of the effect of brain sections on the respiration in the rabbit are accepted, the central nervous mechanism of respiration has important differences in the cat and rabbit. In the cat, the prolonged inspiratory type of breathing is produced by a section which passes dorsally immediately behind the

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STUDIES IN MUSCLE ACTIVITY. I. The static effort.  
By E. P. CATHCART, E. M. BEDALE AND G. McCALLUM.

*(Institute of Physiology, Glasgow University.)*

THE type of activity to which the term static work is applied enters in some degree or other into all forms of muscular effort, and on account of the peculiarly severe strain it imposes on the organism it merits more attention than it has hitherto been accorded. One of us (E. P. C.) has stated elsewhere that, in his opinion, it is the static component of the muscular effort which determines *qua* the organism as a whole whether work is severe or no. The static type of effort may be regarded as the isometric contraction in operation where either the load is of such magnitude that it is beyond the power of the individual to perform external work, e.g. the unsuccessful attempt to move a very heavy weight, or else the weight, being within the individual's compass, is kept suspended by voluntary muscular effort. In the ordinary non-selected type of work, in the industrial sense, it is usual to find the static effort combined with ordinary positive and negative effort. Although the question of the isometric contraction has been fully investigated in a series of researches by A. V. Hill for the isolated muscle, but little attention has been devoted to the problem from a metabolic standpoint on the human subject. Those who have dealt with this form of activity are practically unanimous, and with this we are in full agreement, that the static type of effort is the one which most readily and rapidly induces fatigue.

Chauveau and Tissot (1896-7) may be considered to be the first workers who seriously dealt with the problem. They used the maintenance of loads of varying weight by the flexed forearm as the type of static effort. Although their experimental data are somewhat scanty and the agreement between different experiments is not very satisfactory, they reach the very definite conclusion that when the load is constant the metabolism ( $\text{CO}_2$  output and  $\text{O}_2$  intake) is practically proportional to the degree of shortening of the contracting musculature, *i.e.* to the degree of flexion of the arm, and when the load varies, but with constant degree of contraction, the metabolism is proportional to the load supported. Johansson (1901) also dealt briefly with this type of effort in the course



of his enquiries into the metabolism of muscle activity with his special type of ergometer. He held that the  $\text{CO}_2$  output was proportional to the load. Later, with Koraen (1902) he carried out another series of experiments in which he varied the angle of the contraction of the arms and found that the  $\text{CO}_2$  output increases with the shortening of the muscles. This work was confirmed and slightly extended by Hammarsten (1912). Bornstein and Pohor (1903), in a careful piece of work, emphasise the point that static effort, as compared with dynamic effort, exercises but little influence on metabolism. They suggest that this is due to the rapid onset of fatigue which prevents much "work" being done. Their static effort consisted in supporting a weight with the outstretched arm whilst in the lying position. They came to the general conclusion that the metabolism during the static effort neither increases proportionally to the weight supported nor to the duration of the effort, but in each instance more rapidly. Hellsten (1907) and v. Gerten (1913) investigated the problem simply from a training (practice) point of view. Frumerie (1913) determined the output of  $\text{CO}_2$  in static effort with special reference to the onset of fatigue. He used both the apparatus and methods of Johansson and found that up to 60 seconds at least, the  $\text{CO}_2$  output was directly proportional to the duration of the contraction. He noted that although the subjective sensations have no relation to the amount of  $\text{CO}_2$  given off, there would seem to be a direct relation between the feeling of fatigue and the duration of contraction, also provided no new sets of muscles were brought into play in the attempt to give relief to the active muscles, the sensation of fatigue does not influence the  $\text{CO}_2$  output.

The first real effort to investigate the problem as a whole, however, is found in a paper by Lindhard (1920). The special type of static effort which he utilised was the suspension of the subject's body by hanging by the hands from a horizontal bar. There are at least two drawbacks to this method, (a) the position cannot be maintained for more than a brief period, about a minute, and (b) there is a more or less well-marked fixation of the chest. Lindhard carried out *inter alia* observations on the respiratory exchange before, during and after the performance and found that the maximum output of  $\text{CO}_2$  and intake of  $\text{O}_2$  took place after the cessation of the effort. Although the respiratory frequency reached its maximum during the effort the alveolar ventilation was greatest in the after period. Lindhard also found that the utilisation of  $\text{O}_2$  from the blood was less during the effort than at rest, and that a sharp rise in utilisation occurred immediately on the cessation of the effort, a rise

which was followed by a fall to subnormal value within two or three minutes. He came to the conclusion that these results were due to the fact that static effort, so far as the muscles are concerned, is a type of anaerobic activity. In a later series of observations (1920) he investigated six other types of gymnastic exercise, in which the static effort predominated, and obtained very similar results. It will be noted that in those exercises in which there is least fixation of the chest the post work rise in  $O_2$  intake was least marked. Working in conjunction with Stevenson, one of us (E. P. C.) (1921) found when a weight was maintained in position either mainly by the flexor or the extensor group of muscles that, although the cost of performance was greater than that for negative work, it fell short of the expenditure necessitated by positive work when the load was the same in each type of effort.

In the present series of observations we carried out two types of experiment, (1) in which a load was maintained by the outstretched arms, either forward or backward, whilst the subject lay with the rest of the body as relaxed as possible on a couch, and (2) in which a positive effort was gradually changed into a definite static effort by varying the frequency and duration of contraction of one set of arm muscles against a powerful spring in unit time. The authors of the paper acted as subjects in most of the experiments. The method of gas analysis employed was the usual Douglas-Haldane method; all the analyses were done in duplicate.

### *Continuous static effort.*

In this series of experiments the subject kept suspended by means of a rope over a pulley a 15 kilo. weight by the constant pull of the slightly contracted outstretched arms.

Two sets of experiments were carried out; in both the subject lay in the supine position, but in one the feet pointed towards the weight and with the arms forward (Fig. 1, Position 1), in the other the feet pointed

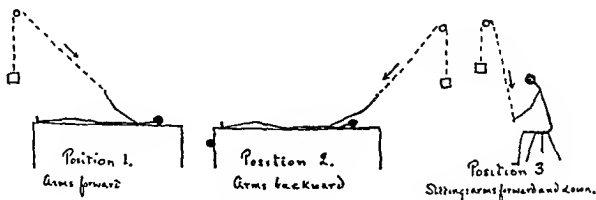


Fig. 1. Positions whilst doing work.

TABLE I. Lying. Static effort continuous. Arms forward. Load: 15 kilo.

Sample	Ventilation litres per minute				CO <sub>2</sub> c.c. per minute				O <sub>2</sub> c.c. per minute				R.Q.				
	i	ii	iii	iv	i	ii	iii	iv	i	ii	iii	iv	i	ii	iii	iv	
Standard		5.55	3.96	5.10	5.18	178	116	184	173	225	153	222	211	.76	.76	.83	.82
Work	1	6.74	5.75	6.02	5.71	214	176	211	180	261	235	252	237	.82	.75	.84	.76
	2	6.88	6.17	6.18	6.32	207	191	206	198	249	228	251	238	.83	.83	.82	.83
	3	—	—	6.03	6.39	—	—	196	209	—	—	236	246	—	—	.83	.85
	4	—	—	6.17	6.32	—	—	202	210	—	—	215	269	—	—	.94	.78
	5	—	—	6.84	6.13	—	—	214	218	—	—	310	280	—	—	.69	.78
	6	—	—	5.43	6.79	—	—	174	234	—	—	229	262	—	—	.76	.89
	7	—	—	11.90	5.57	—	—	296	174	—	—	243	248	—	—	1.22	.70
	8	—	—	—	7.84	—	—	—	242	—	—	—	214	—	—	—	1.13
	9	—	—	—	4.87	—	—	—	147	—	—	—	199	—	—	—	.78
Post work	1	7.14	5.56	3.70	5.00	201	172	118	158	221	197	219	207	.91	.87	.54	.76
	2	5.99	5.07	4.89	5.14	159	153	169	154	209	203	219	211	.76	.75	.77	.73
	3	5.17	5.70	5.49	—	134	173	181	—	189	234	232	—	.72	.74	.78	—

Exps. 1 and 2 by subject B.; 3 and 4 by subject M. Work samples were taken at about 10 minute intervals. First post work sample taken immediately on cessation of work, second, about five minutes later and third about 15 minutes later. Muscle tremor was a marked feature in all experiments.

TABLE II. Lying. Static effort continuous. Arms backward.

Load: i=15 kg. ii=20 kg. iii=25 kg. All experiments on subject B.

Standard	5.16	3.90	6.02	170	123	179	223	141	232	.76	.87	.77	
Work	1	6.15	6.78	8.24	201	193	235	272	257	287	.74	.75	.82
	2	6.45	9.34	9.91	206	274	293	264	304	322	.78	.90	.91
	3	5.94	—	—	186	—	—	245	—	—	.76	—	—
	4	5.85	—	—	189	—	—	239	—	—	.79	—	—
	5	6.40	—	—	197	—	—	237	—	—	.83	—	—
	6	7.18	—	—	238	—	—	261	—	—	.91	—	—
Post work	1	5.02	5.43	6.17	161	143	157	189	193	206	.85	.74	.76
	2	5.32	5.35	5.82	168	172	169	200	230	225	.83	.75	.75
	3	—	5.01	6.03	—	170	186	—	218	229	—	.78	.81

Work samples were collected at about 10 minute intervals. As the subject was very exhausted at the end of pull, the collection of the post work first sample was a few minutes after the cessation of work in Exps. 1 and 2, immediately on cessation in Exp. 3. Other post work samples were collected at about 10 minute intervals.

away and the weight was suspended by extending the arms backwards at an angle of about 30° to the body (Fig. 1, Position 2). The arms were never allowed merely to become passive members in these positions. The weight used could be maintained for a long period without unduly straining the subject, although in the end it was found that in order to keep the weight steady other sets of muscles were gradually brought into play until in the end the total musculature of the body seemed to be involved in the pull. The strain indeed became so intense that in two experiments the end was reached by the subject practically collapsing. The strain came on sooner and lasted longest when the subject kept the weight suspended by lying with the arms extended backwards.

The effect of the effort on the respiratory exchange is found in Tables I and II. In neither the arm forward nor the arm backward position is there any evidence of an increased oxygen consumption during the post effort period, indeed, in six out of the seven experiments, the post effort intake is actually slightly lower than the lying metabolism intake determined immediately prior to the commencement of the effort and in the seventh the basal metabolism is abnormally low. Even in the first after-period of Exp. 3 of Table I, which from the R.Q. might suggest at least a relative increase in  $O_2$  intake, the low quotient is apparently due to the abnormal retention of carbon dioxide.

As regards the arm forward experiments (Table I) it will be noted that the ventilation, in the two short experiments on subject B., is, in both instances, greater in the post effort periods than in the basal, although in one it is less and in the other greater during the period of suspension. In the other experiments on subject M. the ventilation during the first and second post effort periods is lower than in the basal whereas during the periods of effort there is a definite increase in the ventilation rate, very marked in the last period of Exp. 3 and in the last but one of Exp. 4. Possibly the low ventilation recorded in the last period of Exp. 4 was due to impending collapse, although the subject stated that in this period all feelings of impending collapse had passed off.

A consideration of the  $CO_2$  output and the  $O_2$  intake and the ratio which exists between them in Exps. 1 and 2 shows that the effort causes a slight rise in R.Q. due to a relatively greater increase in the  $CO_2$  output, and this is followed in both of the after-periods, carried out immediately on the cessation of the effort, by a very marked increase in the ratio due again to the relatively greater  $CO_2$  output. In Exps. 3 and 4 definite irregular changes occur in the ratio, the maximum being reached with R.Q.'s much above unity in the two periods of maximum ventilation. The subjective sensation of strain, accompanied by more or less convulsive breathing, seems to be associated with these periods of high quotients. These convulsive periods were very intermittent, their disappearance coincided with a drop both in the ventilation and the respiratory quotient.

In Table II, with the arm backward position, all three experiments were carried out with subject B. but with different weights. Although the ventilation during the effort period of all of them was definitely increased, with the two heavier loads very definitely, the  $O_2$  intake is not increased to the same extent as the  $CO_2$  output. These outputs are to a certain degree, comparing the first two periods of Exp. 1 with the two periods in Exps. 2 and 3, influenced by the load. The increase of load

the greatest rise in systolic pressure is 20 mm. Hg, whereas the diastolic is 37 mm., and in the one minute alternation the increase in both pressures is equal at 15 mm.

*Gradually increasing static effort.*

In the final series of experiments a definitely positive type of work which, by mere alteration in timing, became more and more static was utilised. They were carried out by causing the subject to pull on a powerful spring. The muscles involved were mainly the flexors of the forearm although the shoulder muscles also took part in the movement. The spring was pulled through such a distance that each pull was (calculated according to Hooke's law) equal to 1.25 kilogrammetres of work. The operation was carried out as follows: the subject sat down in a comfortable position facing a table on which lay the spring attached to a fixed upright and provided with a suitable grip. At the end of 30 minutes of rest the basal (or standard) metabolism was determined. The cost of the arm movement alone was next determined when the arm was made to move at the set rate for the particular experiment (to the beat of a metronome) and through the proper distance but without touching the spring. Then the work period started when the spring was gripped, pulled out to the appropriate distance; liberated, and the arm moved forward, at a rate identical to that taken during the period of pull, to grip the handle of the spring again, and repeat the operation. Thus, if the rate of work determined on was, *e.g.* four pulls a minute, the metronome was set to beat at the required rate and the operation carried out. The time then taken for each complete movement was therefore 15 secs. and of this period 7.5 secs. were occupied in extending the spring and 7.5 secs. in moving the empty hand forward again to grip the handle. It follows then that in any one minute period of work only half a minute was employed in actual pulling, hence in the most rapid rate of 32 pulls per minute 40 kgm. were done in the half minute and in the slowest rate of one pull per minute only 1.25 kgm. were done in the same time. In all of the experiments the timing was rigidly adhered to and every effort was made to keep the progress of the pull and the return of the arm steady throughout. More rapid rates than 32 per minute were tried but the results, largely owing to interference with respiration, were not satisfactory.

Immediately after the collection of the third sample of the work period (the figures given in Table V are the average of the three periods) was completed, a collection of the first post work expired air was carried out. A second collection was made about five minutes later.

TABLE V Gradually increasing static effort. Spring experiments

Rate	Kg <sub>m</sub> work per min	Subject	Basal		Arm movements		Work, average		(1)		Post work	
			O <sub>2</sub> cc per min	R.Q.	O <sub>2</sub> cc per min	R.Q.	O <sub>2</sub> cc per min	Net cal <sub>s</sub> <sup>1</sup> per min	O <sub>2</sub> cc per min	R.Q.	O <sub>2</sub> cc per min	R.Q.
32	40	B	236	70	292	79	540	1 312	240	85	185	85
		C	246	77	317	82	555	1 190	197	81	159	82
		M	258	77	360	90	633	1 338	358	96	274	82
26	32.5	B	217	70	243	89	522	1 351	244	90	217	70
		C	193	88	290	00	476	922	233	04	192	88
		M	226	79	283	91	490	1 032	250	93	222	77
18	22.5	B	206	81	288	85	457	849	280	04	218	81
		C	201	77	291	90	438	676	225	78	226	78
		M	225	81	231	81	372	674	236	90	145	72
16	20	B	320	70	334	81	510	862	306	78	303	77
		C	276	84	324	87	434	650	211	78	237	88
		M	193	77	276	85	410	674	232	83	200	73
8	10	B	221	75	230	81	331	507	—	—	197	75
		C	—	—	258	90	388	601	237	94	—	—
		M	257	84	294	82	390	183	265	87	280	78
4	5	B	—	—	239	82	327	401	270	73	190	74
		C	—	—	247	91	311	285	235	84	—	—
		M	257	84	290	81	381	439	269	83	213	82
3	37.5	B	272	85	316	93	386	310	230	81	262	83
		C	183	84	229	74	320	434	224	70	181	82
		M	—	—	263	82	320	257	253	72	—	—
2	2.5	B	255	88	284	82	352	313	235	83	—	—
		C	280	84	300	90	363	268	261	81	251	81
		M	197	75	258	73	351	303	283	75	207	69
1	1.25	B	—	—	180	81	252	77	163	70	198	76
		C	—	—	284	82	319	280	—	—	220	86
		M	256	86	—	—	244	124	206	91	—	—
0	0	B	215	70	—	—	—	—	—	—	—	—

First post work observation made immediately on the cessation of work and the second about five minutes later

<sup>1</sup> *I* c less arm movement

TABLE VII. Gradually increasing static effort. Effect of effort on (1) systolic blood-pressure, (2) pulse, and (3) respiration.

Rate	Subject	Basal				Arm movements only				Work 1				Work 2				Work 3				Post work 1				Post work 2			
		Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.	Sys. B.-P.	Pulse Resp.				
32	C	118	69	12	122	70	10	124	74	20	—	90	20	122	100	18	128	80	10	122	70	12	122	70	12	122	70	12	
	C	118	68	14	120	74	9	132	82	16	136	84	19	138	90	20	128	70	18	126	66	14	126	66	14	126	66	14	
	M	112	82	10	118	100	17	122	116	24	126	114	26	126	110	24	120	108	16	112	86	12	112	86	12	112	86	12	
	B	108	76	16	102	88	17	120	88	22	120	108	22	122	116	22	112	86	24	100	84	18	100	84	18	100	84	18	
16	C	114	69	15	122	72	15	130	84	16	134	88	16	138	88	16	128	84	12	120	78	12	120	78	12	120	78	12	
	C	120	64	15	132	72	12	138	76	15	144	78	14	148	76	12	126	66	12	126	66	12	126	66	12	126	66	12	
	M	106	82	11	114	88	16	118	106	17	120	100	19	120	100	19	116	86	18	104	86	16	104	86	16	104	86	16	
	B	109	80	12	116	76	11	130	104	11	128	104	11	—	106	11	124	84	18	114	78	14	114	78	14	114	78	14	
8	C	120	60	13	122	66	13	134	70	18	134	70	16	140	74	16	122	62	11	120	58	14	120	58	14	120	58	14	
	M	100	80	10	109	84	12	111	86	11	124	90	11	114	94	11	106	84	13	109	80	12	109	80	12	109	80	12	
	B	118	80	12	120	92	12	122	102	15	122	108	—	126	104	20	118	100	14	112	88	16	112	88	16	112	88	16	
	C	116	68	14	118	76	10	128	74	8	132	76	12	132	80	8	124	70	8	120	66	12	120	66	12	120	66	12	
4	M	116	80	10	116	96	12	121	94	16	121	94	16	121	94	16	110	86	12	107	83	15	107	83	15	107	83	15	
	B	120	76	10	120	84	12	122	92	12	130	100	12	—	—	—	124	82	12	120	80	12	120	80	12	120	80	12	
	C	118	62	12	120	66	12	125	66	12	130	70	12	—	—	—	124	62	8	117	57	10	117	57	10	117	57	10	
	M	114	88	12	116	96	16	122	96	16	124	98	12	—	—	—	120	86	—	113	84	14	113	84	14	113	84	14	

The various observations were made during the time of the arm movements in the basal determination and in the work determination. The first post work observation was made immediately on the cessation of work and the second observation five minutes later.

definitely influenced in the rapid series but the acceleration during the slow movements is not marked.

As regards the sense of fatigue there can be no hesitation in stating that, in common with most other observers, we found the degree and duration of the fatigue induced by the static effort to be out of all proportion, either to the amount of work done or to the duration of the experiment. Frumerie (1913) holds, as already mentioned, that there is a direct relation between the sensation of fatigue and the duration of the contraction, so long as the load remains constant. He also believes that the sensation of fatigue is due to mechanical pressure on the nerve endings in muscle. Lindhard has suggested that the static fatigue is due to actual fatigue of the muscle substance. Two facts in particular would tend to show that in this static fatigue we are dealing with another phenomenon to the fatigue induced by prolonged positive work, viz (1) the fatigue induced is definitely associated with pain, and (2) tremor of the muscles employed comes on very rapidly, a tremor which may last for many minutes up to, at least, a couple of hours after the static effort has ceased. The duration of the after effect depends, of course, largely on the duration and the severity of the static effort. In the continuous experiments (Tables I and III) tremor was a very conspicuous feature.

#### SUMMARY.

(1) Three types of static effort were investigated, (a) continuous, (b) intermittent, (c) gradually increased.

(2) In these types there is no evidence to support the view that there is a reduced intake of oxygen during the effort.

(3) There is also no marked increase in the oxygen intake after the cessation of the effort.

(4) There is but little alteration of the respiratory quotient

(5) There is, as a general rule, a definite rise in the pulse and respiration rates and in the blood-pressure during the effort.

(6) The increase in diastolic is more marked than the increase in systolic pressure.

(7) In an effort which is gradually changed from being definitely positive to being definitely static there comes a rate of performance when the cost per kgm. rises and continues to rise as the effort becomes more static. The rise in cost is associated with a marked decline in the mechanical efficiency with which the effort is made.

(8) Fatigue is a very pronounced feature of static effort.

The greater part of the expense incurred in connection with this investigation was defrayed by the Medical Research Council, to whom our thanks are due.



is slightly concave. It should project a very tiny spot of light upon the vertical slit not more than 1 mm. in diameter. The mirror moves upon

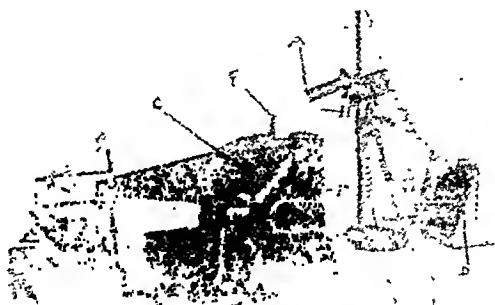


Fig. 1.

a horizontal axis and is placed in connection with the artery by a method shortly to be described. The sensitised paper is set in position by an electromotor (*D*) which is hung from the ceiling, and is connected with a grooved wheel by an elastic band. In the axis of this wheel is a wormwheel which sets in motion within the instrument a vertical rod between which and another rod the sensitised paper is pulled along. The electromotor being first started, the whole is set in motion by a friction-clutch (*E*), and the speed at which the paper travels can be regulated not only by shifting the elastic band to other positions in the vertical wheel, but by a change-gear within the instrument. After a tracing is taken the paper may be cut by a vertical knife (*F*), and the rest of the instrument detached and removed to the dark room. Two very small electric lamps are each of them covered by metal, except for a hole through which a spot of light is projected into the lower part of the vertical slit. One of them is connected by wires (*H*) with a Brodie's time marker, and gives a spot of light upon the paper every two seconds or less, and the other is arranged in connection with an induction-coil, and gives a horizontal line which corresponds with the time in which a nerve may be stimulated.

The mirror (Fig. 2 b, *M*) is fixed to the centre of a light metal plate, which rotates upon a horizontal axis; it is fixed so that its anterior surface is in a line with this axis. At either end of the plate in this axis is a little cup of metal in which fit the tiny screws (*E*). The mirror can then swing quite freely. Behind the mirror is a groove of metal in which slides a tiny slip of metal (*C*) which is bent below

and forward nearly to a right angle (*D*) and rests upon the artery (*A*). The pressure of this upon the artery is only a few milligrammes, but in

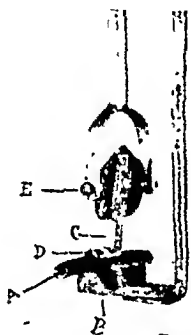


Fig. 2a.

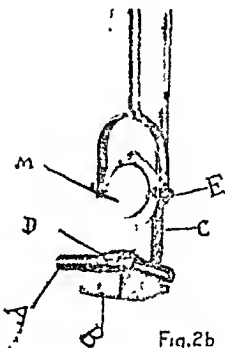


Fig. 2b.

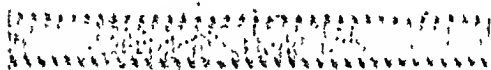


Fig. 3.

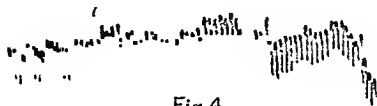


Fig. 4.

order that this weight should be more distributed, another piece of metal, about a quarter of an inch long, is fastened across it at right angles. This is curved so as to fit upon the artery. Before applying this to the artery it is smeared with thick vaseline in order to prevent it from being thrown upwards after each systole of the heart. By means of the groove behind the mirror it is possible to regulate the movements of the mirror and to make them greater or smaller.

In the case of the abdominal aorta of a cat (anæsthetics, A.C.E. and phenaldehyde) it is not necessary to remove the vessel from its attach-

ments; the plate in connection with the mirror may be placed directly upon it; this is the easiest way to take tracings.

In the case of the carotid the respiratory movements of the neck are much bigger and interfere with the result. It is necessary to isolate the artery for about two inches, and to place it upon something which is immovable. The artery is therefore placed in a groove of a piece of ebonite which is supported also upon an upright; it is important that the artery should lie in a normal position. By bending the head (an additional joint was put into the rabbit-holder), this is brought about and the artery can then be placed in position upon the ebonite plate so as to lie in the same line with it.

The metal rod holding the mirror can be arranged upon it, and it can be moved by screws up and down, from side to side and from before backwards. These screws are not seen in the figure. It is most important to work with two tables, one for the kymograph, and the other for the animal, the support for the mirror, and the ebonite plate. The reason is that the vibration of the kymograph would otherwise shake the animal, and the mirror attached to it. In Fig. 1, they are both covered by a sheet for purpose of photography. The aperture in the photographic kymograph through which the vertical slit is seen is covered by a piece of ruby glass. The sensitised papers (Nikko) Kodak Co., are 10 or 5 cm. wide and can be prepared in lengths of 100 yards or more. I have to thank Mr J. Brown for very kind help in preparing them. While the vaseline holds the mirror to the artery of a rabbit and a cat, in the dog the movements are so rapid and jerky that this is not the case, and the results will be dealt with in another paper.

In order to determine whether the inertia of the mirror would throw it away from the artery, it was attached to a tuning fork vibrating 25 times to the second, by means of vaseline. The tracing obtained showed an even curve (Fig. 3). Of course the movement of the tuning fork must be small otherwise the mirror would at once fly off.

As Ray was I believe the first to point out, the expansion of an artery is not a linear measure of the pressure of the blood within it. One cannot therefore record the absolute pressure, but one can safely say that at one portion of the curve the pressure is higher or lower than at another, which is all that one can say for a Lüdwig's or a Fick's curve.

*Tracings obtained.*

Fig. 4 shows the normal blood tracing in the rabbit; the tracing could of course be obtained much larger. It will be seen that the respiratory movements are small while those of the artery are large. In many other cases it is hard to see that there are any respiratory movements at all. The variation in the thickness of the lines of course indicates the rate of movement of the artery.

Fig. 5 shows two curves obtained by stimulating the peripheral end of the vagus. The lower straight lines indicate the duration of the stimulus. The dots indicate time  $\frac{1}{10}$  second. This is like the curve for the mercury manometer except that after the stimulation it does not exceed the upper level of the curve.

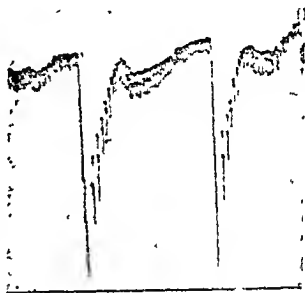


Fig 5.

The difference may probably be due to the flow through the artery being obstructed in the case of the mercurial manometer, and free in the other. Another point in which the tracing differs from that obtained by the mercury manometer is the very much larger curve obtained for each heart beat. The curve obtained by stimulating the depressor nerve is like that given by a mercury manometer except of course that the variations in size due to the heart beats are much more extensive.

Fig. 6 shows the tracings obtained by the instrument adapted for the frog's heart; another and much longer piece of metal is placed in the groove at the back of the mirror. There is of course no need of the cross curved piece which is essential for the artery.

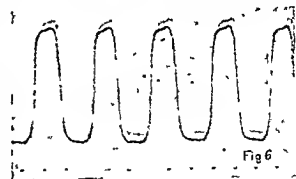


Fig 6

I have to thank most heartily Professor Langley for the generous manner in which he adapted a room in his laboratory for my work.

I have to thank my former mechanical assistant Mr Josiah Miles for the care with which he constructed most of my apparatus.

I have finally and not less warmly to thank the Medical Research Council for their generous gift in aid of the research, a portion of which is here published.

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The perfecting of the instrument described in the foregoing pages had long been close to the heart of Prof. Haycraft. He considered that the existing methods of recording variations of arterial pressure gave a very inaccurate rendering of the actual variations and he hoped to fashion an instrument giving a true record. The short account he has given was to be the forerunner of a full investigation. He had obtained a number of tracings from the arteries both of man and animals, which showed no dicrotic wave and he believed that this wave was artificial—the result of narrowing the lumen of the artery by pressure. Whilst engaged in testing the trustworthiness of the tracings, death struck him down, and his work is left for others to complete.

J. N. L.

# ON THE SITUATION AND EXTENT OF THE PURELY YELLOW ZONE IN THE SPECTRUM.

By GUSTAF FR. GÖTHLIN, *Upsala*.

NEWTON, the first to investigate the spectrum in detail, also determined the limits of the spectral colours laid down by him(1). What Newton characterised as yellow in the spectrum is undoubtedly the whole of the region that makes a predominantly yellow impression on the eye, consequently including that part of the yellow-green in which yellow predominates. According to the calculations of Fresnel(2) in 1825 as to the wave-lengths of the beams that correspond to Newton's limits between the spectral colours, the wave-length limits for yellow lie at  $532\mu\mu$  and  $571\mu\mu$  respectively. By means of an improved method of calculation, Drobisch(3), in 1853, corrected these values to  $537.7\mu\mu$  and  $588.6\mu\mu$  respectively. In 1860, Clerk Maxwell(4), on the basis of numerous colour-mixture equations, made out curves for the fundamental colours in the spectrum. The point of intersection between his curves for red and green is not in complete agreement in his two graphic representations of two series of experiments; but it lies approximately at the wave-length 2116 according to Fraunhofer's system, corresponding to  $572.8\mu\mu$ . Here, therefore, according to Maxwell's measurements, one expects that the purest yellow will lie. Listing(5) states that pure yellow corresponds to a wave-length of  $559\mu\mu$  and places the limits between yellow and green at  $534.7\mu\mu$  and between yellow and red at  $585.6\mu\mu$ . This figure of Listing's for the limits of yellow is still quoted in the last edition of the tables of Landolt and Börnstein(6). In his description of the spectrum Helmholtz(7) states that there is a very small line (Strieh) of pure yellow which is about three times as far from *E* as from *D*. It does not appear quite clearly from the context whether it is in the normal spectrum or in the prismatic spectrum that Helmholtz considers the position of the yellow to be stateable in this fashion. If the normal spectrum is intended, that would mean that the wave-length  $574$  ( $573.7$ )  $\mu\mu$  is in the middle of the yellow. If, on the other hand, the prismatic spectrum is intended, a somewhat shorter wave-length would represent the mean wave-length of that light which is conceived as pure yellow in the spectrum.

F. C. Donders(8) caused 76 persons to fix the position of their *purest*

yellow in the spectrum. The extreme lights which any eye amongst the persons examined by Donders fixed upon as purest yellow had the wave-lengths of  $572\mu\mu$  and  $594\mu\mu$  respectively, and the mean value of the determinations of all the eyes as regards the purest yellow was  $582\mu\mu$ . E. Hering<sup>(9)</sup>, according to whose theory yellow is a fundamental colour in the meaning that the sensation so named can be produced by a unitary process, contested Donders' conclusion that in different persons light of different wave-length was seen as the purest yellow. He held that the sensation of purest yellow was produced in different persons by light of the same wave-length. K. Schaum<sup>(10)</sup> essayed a division of the predominantly yellow zone in the spectrum. He divided (p. 65) the spectral yellow of the physicists into golden yellow, yellow and greenish-yellow. Starting from the Fraunhofer lines *C*, *D* and *E*, the extent is given in the tables as follows:

For golden yellow	$C \frac{2}{3} D - D \frac{1}{3} E$ (i.e. $606-578.9\mu\mu$ ) <sup>1</sup> ;
For yellow	$D \frac{1}{3} E - D \frac{2}{3} E$ (i.e. $578.9-568.5\mu\mu$ );
For greenish-yellow	$D \frac{1}{3} E - E$ (i.e. $568.5-527\mu\mu$ ).

I have not been able to find any previous experimental investigation made with the object of determining the extent of *pure yellow* in the spectrum. As it seems that the knowledge of this would be of both theoretical and practical importance, I have tried to make good the gap. The investigation has been carried out on 24 persons, the majority of whom have often been obliged to judge of colours in connection with their daily work.

*Methods.* The testing has taken place with monochromatic lights which have been shown in the writer's liminospectroscope. The design of this instrument has been described in detail (11) in the *Transactions (Handlingar) of the Royal Swedish Academy of Science*, Vol. 58, I, 1917. A careful wave-length scale for the instrument within the spectral region that was to be dealt with—that is to say, the yellow and immediately adjacent parts of the spectrum—was first worked out. By the use of a heliostat in a room with greatly subdued light, certain Fraunhofer lines were brought, with the aid of the Gauss-ocular of the apparatus, into the middle of the slit of the telescope tube. This slit had been beforehand regulated to a width of 0.2 mm. At the same time the width of the slit

<sup>1</sup> The calculation of the wave-lengths corresponding to the original figures has been worked out by the present writer on the assumption that the figures refer to the normal spectrum, a matter, however, on which the original work gives no information. On what investigations the figures given by Schaum rest is not stated. In naming the colours he followed Helmholtz.

of the collimator tube was 0.05 mm. The standard lines sought out were the *C* line, the middle of the interval between the lines  $D_1$  and  $D_2$ , and line  $b_1$ . For each line five completely independent determinations were carried out, after which the mean value of these was taken. In this way it was established that the *C* line (6563 Ångström units) corresponded to the scale-line marked 5594 on the revolving mechanism of the prism; the middle of the interval between  $D_1$  and  $D_2$  (5893 Å. units) corresponded to 5185 on the scale; and the line  $b_1$  (5184 Å. units) corresponded to 4500 on the scale. With the help of Hartmann's (12) formula it was deduced for the region between *C* and  $b_1$ , that

$$S = 7469.9 \frac{7021475.5}{\lambda - 2820},$$

where  $\lambda$  is the wave-length, expressed in Ångström units, which it is desired to sight, and *S* is the numerical value of the corresponding line on the revolving mechanism of the prism. The correctness of the formula was verified on the lines  $\alpha$  and *E*, the situation of which had been directly established in precisely the same way as the above given standard lines, and also on the thallium line. With regard to these lines chosen as samples, the use of the formula led to values which were in complete accordance with those read off in the direct determinations of the lines. On the basis of the formula there were then calculated and set up in tabular form the values of *S* for all wave-lengths between  $630\mu\mu$  and  $520\mu\mu$ , amounting to multiples of  $1\mu\mu$ . As a rule I have contented myself with showing beams with a difference of  $1\mu\mu$  in wave-length.

The liminospectroscope, in which the source of light is a Nernst slit lamp of Gullstrand's design (13), is set up in such a way that the source of light, the illumination tube with its polarisation arrangement, and the prism table with its revolving mechanism and scale are in a dark chamber, while the telescope tube penetrates a wall impervious to light leading to another dark chamber in which sits the person who is being experimented on.

The prism in the apparatus has a dispersion of  $5^\circ 30'$  between the Fraunhofer lines *C* and *F*. In all the main experiments the work has been carried out with a slit of 0.03 mm. in the collimator tube and a slit of 0.3 mm. in the telescope tube. With this combination of slits I have determined the spectral breadth ( $d\lambda$ ), expressed in wave-lengths, in the light going out through the slit of the telescope tube and found it amount to  $0.38\mu\mu$  for light of wave-length  $572.5\mu\mu$ , to  $0.42\mu\mu$  for light of wave-length  $582.5\mu\mu$ , and to  $0.45\mu\mu$  for light of wave-length  $592.5\mu\mu$ . Consequently one must take into account the fact that, if one sights a



wave-length of  $580\mu\mu$  for instance, one finds in the emerging beam also wave-lengths that exceed and fall short of  $580\mu\mu$  by about  $\frac{1}{4}\mu\mu$ .

In the chamber where the observer is installed, above the telescope opening of the liminospectroscope, there is fixed a fairly large pasteboard screen, coated with magnesium oxide. The screen slopes somewhat towards the chamber with its upper edge. On a stand near the opposite wall, that is to say, behind the back of the person under experiment, is placed the source of light, which determines the nature and strength of the illumination upon the screen.

As there does not yet exist any international agreement as to what should be regarded as "white light," I confined myself to considering as white or neutral, from the standpoint of colour, the illumination on the screen from a sky, which was free from clouds except an even and uniform covering of cirrho-stratus. Neither the sky nor the magnesium oxide screen seemed under these circumstances to exhibit any colour that was observable to the eye. During a day when the whole sky that could be viewed from a window freely facing the south-west was covered by such cirrho-stratus, the magnesium oxide screen just mentioned was placed at a distance of one metre inside and at an angle of  $45^\circ$  to that window, so that it was entirely illuminated by the light from the sky. Directly opposite the screen was set up a Weber photometer, the observational tube of which was levelled against the screen. In the line of the other tube, which is generally occupied by the source of light of the photometer itself, there was placed a half-watt lamp of 75 candle-power in a light-proof box in such a way that the connection between that box and the photometer tube was impervious to light. In the light-proof space between the half-watt lamp and the photometer-tube there was set up first a glass trough with plane parallel walls, intended to hold a solution of sulphate of copper, and secondly, a frame with several partitions intended to hold on the side nearest the tube a piece of ground-glass and beyond that, if necessary, green or blue glass filters.

After the illumination in the two fields of the Lummer-Brodhun biprism of the photometer had been made of uniform strength by first putting a suitable ground-glass in the frame in the front of the half-watt lamp and afterwards by moving the movable ground-glass in the graded tube of the photometer, the tone of colour in the field which received its light from the half-watt lamp was changed by means of filters until the eye could not discern any difference between that light and the neutral light which the other part of the field for comparison received from the magnesium oxide screen illuminated by the sky. During these operations

it was not necessary to make use of the blue and green glasses which were held in readiness, for what was to the eye complete identity in tone of colour was in practice obtained at a certain concentration of the solution of sulphate of copper in the trough.

The neutral artificial source of light thus obtained, consisting of a half-watt lamp in its light-proof box, the sulphate of copper trough—the contents of which had previously been cut off from access of air by pouring a layer of melted paraffin over the liquid—and finally, the ground-glass, were then moved on to the stand in the dark chamber for the observers. The magnesium oxide screen was also brought into position in the way that has already been stated. A definite point of fixation was selected on the screen and was indicated by a ring-shaped mark, which was also of cardboard covered with magnesium oxide. Finally, so much of a piece of black opaque cardboard was inserted in front of the ground-glass in the artificial source of light that, if a Weber photometer was applied to the place of the observer and its observation-tube was levelled straight at the fixation mark on the screen, and at the same time a plate of ground-glass in the mouth of the tube was in the same place that the eye of the observer was afterwards to take, the total illumination on the place intended for the eye amounted to one metre lux. To this illumination all the persons experimented on had to adapt themselves first, at the beginning of the experiment.

The exposures in the apparatus did not begin until the person under experiment had looked at the mark on the screen for 10–12 minutes continuously, and the definitive determinations did not take place until about half an hour later. Thus, the organ of sight had been adapted for an illumination of one metre lux at the place for the eye for about 40 minutes, although there were interruptions for the purpose of taking readings. It may, perhaps, be objected that it would have been more thorough-going and better to cause the adaptation to take place for total darkness. But in practical life one does not judge colours with eyes adapted to total darkness. Moreover, if a long period of adaptation to total darkness had had to precede the judgment, difficulties would have arisen in the way of getting hold of a sufficient number of qualified persons for the investigation.

The persons subjected to my experiments were partly artists exercising the art of painting, partly persons who had successfully devoted themselves to painting in the capacity of amateurs, and finally, in some cases persons (physiologists, psychologists and women-teachers of drawing), who had proved themselves to have a good eye for colour without belonging

In this test, as in most of the others, the wave-lengths of the beams shown have formed whole multiples of  $1\mu\mu$ . If the results are expressed without considering the actual spectral breadth ( $< 0.5\mu\mu$ ) of the several beams, G. J.'s pure yellow will correspond to the wave-lengths 580-584  $\mu\mu$ .

As the construction of the liminospectroscope readily admits of producing beams with wave-lengths that differ by only  $\frac{1}{2}\mu\mu$ , although in such cases with the same spectral breadth as if they differ by  $1\mu\mu$ , the former method of testing has been employed in some cases. In one individual (No. 8) it was even necessary to have recourse to this expedient, because the observer's zone of pure yellow was found to have a breadth falling short of  $1\mu\mu$ .

Without giving complete records of every examination, I give below, on basis of the records, what wave-lengths those beams have which are conceived as pure yellow by the various persons experimented on under the conditions prevailing at this investigation:

Observers: <i>women</i>							Yellow zone $\mu\mu$
1.	G. J., painter	...	...	...	...	...	580 -584
2.	M. L., portrait-painter	...	...	...	...	...	580 -581
3.	E. B., "	...	...	...	...	...	577 -582
4.	E. H., painter	...	...	...	...	...	579.5-581.5
5.	M. A., drawing teacher	...	...	...	...	...	579 -581
6.	I. A., assistant for scientific drawing and painting	...	...	...	...	...	581 -589
7.	Gr. S., "	...	...	...	...	...	583 -584.5
8.	M. Sj., amateur painter	...	...	...	...	...	584 -584.5
9.	I. G., "	...	...	...	...	...	578 -581
10.	E. Ö., "	...	...	...	...	...	578 -583
11.	B. H., "	...	...	...	...	...	578 -580
12.	H. A., "	...	...	...	...	...	582 -587
Observers: <i>men</i>							
13.	J. Ö., painter	...	...	...	...	...	587.5-590
14.	L. Ij., scientific painter, lithographer	...	...	...	...	...	577 -583
15.	E. B., medical doctor, amateur painter	...	...	...	...	...	583 -585
16.	N. Z., university lecturer, amateur painter	...	...	...	...	...	577 -582
17.	Fr. Sj., university graduate, amateur painter	...	...	...	...	...	574 -585
18.	Hj. Ö., professor emeritus in physiology	...	...	...	...	...	578 -583
19.	B. H., professor of teachers' training, psychologist	...	...	...	...	...	588 -596
20.	S. A., lecturer in psychology	...	...	...	...	...	589 -591
21.	G. G., professor of physiology	...	...	...	...	...	584 -591
22.	L. B., demonstrator in physiology	...	...	...	...	...	583 -584
23.	C. G. S., physician, physiologist	...	...	...	...	...	579.5-586.5
24.	B. C., assistant in physiology	...	...	...	...	...	579 -584

A more rapid survey of the individual variations, with regard both to the position and to the extent of the pure yellow zone, can be obtained by a glance at the accompanying graphic representation of the results (Fig. 1).

Thus the test shows that, under identical conditions of experiment, the purely yellow zone in the spectrum for normal organs of vision have

lain at different ranges of wave-length, *e.g.* for observer No. 14 at 577–583  $\mu\mu$ , for No. 15 at 583–585  $\mu\mu$  and for No. 13 at 587.5–590  $\mu\mu$ . This result is irreconcilable with Hering's unproven assertion that in a

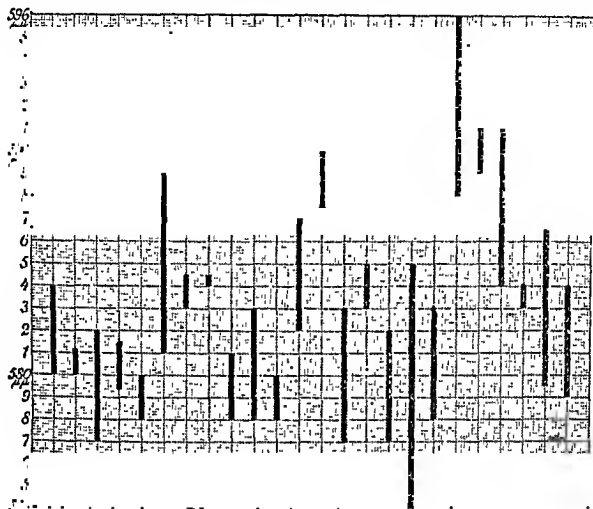


Fig. 1.

neutral condition of the eye the pure sensation of yellow ("Urgelb") in the central vision in different eyes is produced by one and the same radiation. Nor can that radiation which is characterised by Hess(14) as invariably yellow (with a wave-length of 574.5  $\mu\mu$ ) be regarded as other than individually valid. To my own organ of sight, as to that of observer No. 22, for instance, the radiation with a wave-length 574.5  $\mu\mu$  on direct fixation makes the impression of a yellow greatly mixed with green; and it undergoes a very appreciable change in the tone of colour when the image is moved out to the intermediate zone of the retina. Probably, therefore, the yellow which is characterised by Hess as invariable is also subject to individual variations in the matter of wave-length.

A glance at the table on p. 188 and Fig. 1 shows that there does not exist any monochromatic radiation which makes the impression of pure yellow on all normal organs of vision that are in a neutral condition and have been adapted to such a faint illumination as one metre lux, when the

to-day in persons suffering from what is called green blindness—an anomaly which, therefore, I conceive as a kind of atavism. In the intermediate retinal zone, and also in the central zone of those suffering from green blindness, it is sufficient to assume a white-process, a blue-process and a yellow-process. During the evolution from this dichromatic condition to the highest development of colour vision, I suppose<sup>1</sup> that, by degrees, a red-process and a green-process have been substituted for the simple yellow-process and that the red- and green-process respectively, when alone, elicit a sensation of red or green, but when simultaneously present in a definite relative strength result in a sensation of pure yellow.

The condition in the intermediate zone (I) and the condition in the central zone of the normal eye, when the yellow-process has been entirely replaced by the red- and green-process (II) according to this hypothesis can be represented schematically in the following diagram. Yellow, which

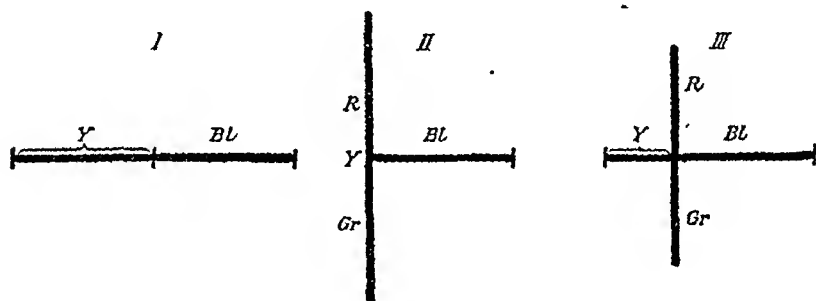


Fig. 3.

in I represents about half the spectrum, dwindles in II down to a point at the transition of red in green. Between I and II intermediate states (III) must be supposed, characterised from the fact that a remainder, greater or less, of the simple yellow-process yet exists along with its derivatives, the red- and green-process. The explanation of the different breadths of the yellow zone in different individuals is self-evident with this working hypothesis. In the case of severe restriction in the yellow zone, as in the case of observer No. 8, it is to be assumed that the yellow process in the central range of vision has been completely replaced by the green- and red-processes. In cases where the purely yellow zone has a greater spectral breadth one must assume a corresponding remainder of undifferentiated yellow-process in the central range of vision also.

According to the cleavage hypothesis mentioned above, a normal sense of colour in the central part of the retina (a field of 2°) would be

<sup>1</sup> This hypothesis has certain points of resemblance to one put forward by Chr. Ladd Franklin (15), but is less fully specialised.

in certain cases, and those the furthest advanced, from the point of view of historical development, trichromatic in the sense that only three chromatic elementary processes occur there (the blue-process, the green-process, the red-process), while in other cases it would be tetrachromatic in the sense that four chromatic elementary processes (blue-, green-, red-, yellow-) exist alongside of one another.

Starting from the hypothesis that a certain amount of the yellow-process becomes divided, when colour-vision becomes more highly differentiated, into equivalent amounts of red- and green-processes, the explanation of the individual dissimilarities with regard to the position of the yellow zone may assume something like the following shape.

*First alternative.* The green-process and the red-process are not elicited in all neutral eyes in the same proportion on illumination with a given monochromatic light. This alternative presents itself, for instance, if in some eyes—namely, those with a yellow zone of relatively long wave-length—the substratum for the red-process were found in a smaller quantity in comparison with the quantity of substratum for the green-process. The same alternative might also be realised to a certain extent in another way, namely, that the fluorescence effects of the eye media and the retina were of different strength in different eyes. If, for instance, these formations, in the presence of radiation of the approximate wave-length of yellow (about  $580\mu\mu$ ), presented in certain eyes a stronger fluorescence effect according to Stokes' law than in others, the position of the purely yellow would thereby be moved by the fluorescence effects in the direction of shorter wave-lengths in eyes of the former kind.

The *second alternative* in the way of explanation would be this: a radiation of a given wave-length brings about in all eyes in a neutral state the same relation between the intensity of the green-process and that of the red-process in the retina, but this relation is changed in some persons on the way to the centre of vision either through more unfavourable conditions of transmission or through a lower susceptibility in the centre itself for one of these processes than for the other. According to this alternative explanation it would have to be assumed that those whose yellow zone is seriously shifted to the red side of the spectrum, have an inferior transmission power or a lower central susceptibility for the red-process than for the green-process.

#### SUMMARY.

The situation and extent of the purely yellow zone in the spectrum was investigated by experiments on 24 normal trichromats accustomed to judge colours, 12 of either sex. At the time of the examination, their

eyes were adapted to a neutral light corresponding to an illumination of one metre lux at the place occupied by the eye. The spectral breadth of the monochromatic radiations with which the tests were made, fell short of  $0.5\ \mu\mu$ .

Under these conditions it was established that both the situation and the extent of the purely yellow zone in the spectrum varies from individual to individual (cf. Fig. 1). For instance, in one person (No. 14) it lay at  $577\text{--}583\ \mu\mu$ ; in another (No. 15) at  $583\text{--}585\ \mu\mu$ ; and in a third (No. 13) at  $587.5\text{--}590\ \mu\mu$ .

On examining these 24 persons no radiation with greater wave-length than  $596\ \mu\mu$  and no radiation with shorter wave-length than  $574\ \mu\mu$  was conceived as pure yellow.

The spectral extent of the purely yellow zone varied in different eyes between 1 and  $12\ \mu\mu$ , and was, on the average, less in the women experimented on (mean figure,  $4.1\ \mu\mu$ ) than in the men experimented on (mean figure,  $6.1\ \mu\mu$ ).

The radiation which received the largest number of votes (14 out of 24), as belonging to the purely yellow zone, was a radiation with the wave-length of  $580\ \mu\mu$  (cf. Fig. 2).

The results of the investigation make it evident that no monochromatic light exists which makes the impression of being purely yellow on all normal trichromatic organs of vision which are adapted to a weak (one metre lux) neutral illumination, when the radiations exposed are not chosen stronger than is necessary to render possible the distinguishing of colours.

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## A SIMPLE APPARATUS FOR PRODUCING MECHANICAL HEMOLYSIS AND ITS PRAC- TICAL USE. BY D J DE WAARD

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of Groningen, Holland)*

For several purposes it is desirable to hemolyse blood without addition of chemicals. It has been done by freezing and thawing repeatedly, by electric discharges, by shaking with mercury, glass-powder<sup>(1)</sup>, asbestos fibres<sup>(2)</sup>, or by rubbing with sand<sup>(3)</sup>. These methods take considerable time and do not guarantee complete hemolysis. A very good method is by forcing blood intermingled with infusoria earth and packed in a layer of cotton-wool, through a Buehner press<sup>(4)</sup>. This, however, can only be done with considerable quantities of blood and some loss. Further there exists a possibility of constituents of blood being adsorbed by the infusoria-earth, so that the resulting fluid may inaccurately represent the original composition of the blood. In order to avoid these drawbacks I constructed a small apparatus with which blood can be hemolysed completely and by purely mechanical means. It depends upon the principle of forcing blood under a high pressure through a very narrow slot.

In the dairy technique, machines depending upon this principle have long been used. Barthel discovered that the fat globules could be reduced in size by mechanical influences. Julien built a machine in 1892 by means of which milk was forced through narrow openings under a pressure of 250 atmospheres, in which he caused the resulting spouts to clash against each other. In 1900 Gaulin built a machine by means of which milk was forced under a high pressure into a tube closed with a cone of agate, which was kept in the opening with a strong spring. After Gaulin many others constructed machines dependent upon much the same principle. With most of them the fat globules are reduced to the limit of microscopical visibility.

According to this principle a simple apparatus can be made for hemolysing blood as follows (Fig 1).

A thick walled brass tube (*a*) of 8 mm bore is provided on one side with a cross bar (*R*). On this side the wall of the tube is turned out in a conical shape at an angle of 45° to the axis. In the two sides of the cross bar a screw hole is cut, through which a bolt (*S*) runs. The bolt passes



at the distal end through a hole of a bar (*T*) of the same length as the cross-bar and at the end it is provided with a nob. A steel spiral spring

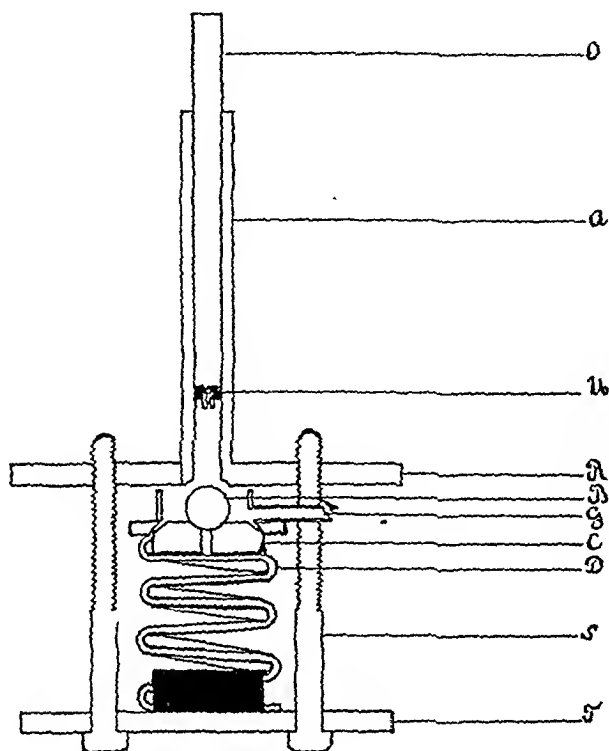


Fig. 1.

(*D*) when compressed by the bolts presses on to a holder (*C*), which presses a steel ball of  $\pm 12$  mm. (*B*) in the conical opening. Such a spiral spring must be used as will be shortened to half its length by 50 kg. In the tube there is a piston consisting of a steel piston-rod (*O*) of 7 mm. diameter, provided with a leather bucket (*U*) fastened with a small screw and nut to the rod. The piston should be soaked in water before being used in order to fit.

To make the ball fit exactly it is put on the conical-shaped opening, while the other end of the tube rests on an anvil, and struck two or three times with a rather heavy hammer. The result is that the hard steel ball forces a bed in the opening of exactly the same curvature as that of the ball. After this the ball-holder (under which the ball lies) is pressed by means of the bolts and the spring. A certain pressure put on the interior of the tube will produce a very narrow slot between ball and wall of the

tube through which the contents can escape. When the tube is filled with blood and the piston is pressed into it with a strong screw, there will escape through the slot-laked blood, in which only sporadic un-hemolysed erythrocytes are to be found. (The pressure at which the blood escapes should not be less than 60 atmospheres.)

The following phenomena may be observed by using this apparatus.

If blood that is made uncoagulable by a citrate is forced through this press we get a clear liquid that will coagulate after some time. Whether this coagulation is caused by a certain quantity of Ca that is to be found in the erythrocytes, by a change in the acidity of laked blood with regard to the plasma through the contents of the erythrocytes, or by particular substances, is not settled and will further be examined.

Unprepared blood not yet coagulated will coagulate after being forced through the press. Instead of the blood itself we can also take erythrocytes from which the plasma is removed by centrifuging defibrinated blood, so that we get the contents of the erythrocytes with very little serum.

If this instrument is used for horse's blood, the property of horse's hemoglobin crystallising very easily is disclosed. In contact with the air and especially with oxygen the resulting liquid contains a great many hemoglobin-crystals. This spontaneous crystallising is probably owing to the lesser solubility of oxyhemoglobin in contrast with reduced hemoglobin at room temperature(5). In this connection it may be noted that Hamburger saw hemoglobin-crystals in erythrocytes. If we wash horse's erythrocytes with a hypertonic solution of sugar by means of which we draw water from them, we obtain after forcing them through the press a half solid crystalline substance that can very well be used for preparing hemoglobin.

With this simple apparatus 50 c.c. of blood can be easily hemolysed in half an hour (the contents of the cylinder is 5 c.c.). If larger quantities are required we can take a wider and longer tube, but in that case the spring must be proportionately stronger. In this manner the apparatus will lend itself to obtaining laked erythrocytes that may be used for all kinds of physical-chemical researches.

I may describe here the possibility of another use, namely determining refractometrically the hemoglobin-content of blood according to Howard(6). In order to make this determination fit for clinical use it is however necessary to reduce the quantity of blood to a minimum, in other words to make the contents of the pressing tube as small as possible, so that a few drops of blood will be sufficient. For a narrow pressing-tube

it is almost impossible to make fitting pistons, therefore the apparatus has been constructed a little differently, as follows (Fig. 2. A and B).

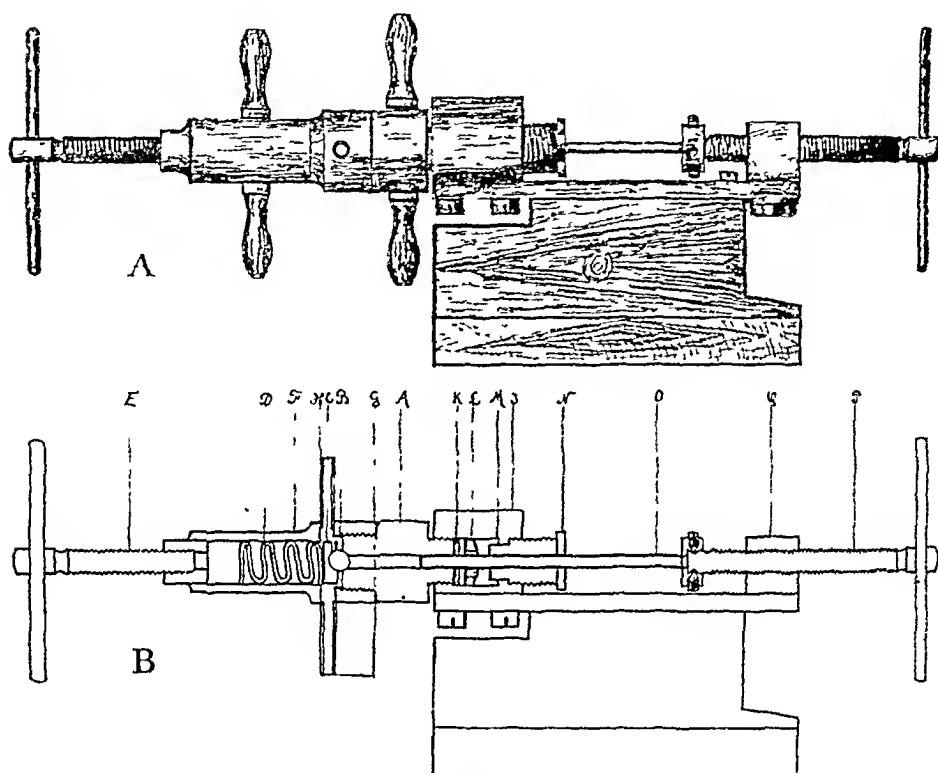


Fig. 2.

The apparatus consists of a piece of brass *A*, 4 cm. long, with a cylindrical bore of 4 mm. One end of the bore is turned out conically under an edge of 45°. A steel ball *B* of 10 mm. is first hammered and then pressed in this opening by means of a brass holder *C*, a spring *D* and a screw-bolt *E*. The brass tube *F* is screwed to *A* and has a little tube *G*, through which the hemolysed blood can drain; sometimes it may be necessary to blow into the instrument by means of tube *H*. The piece *A* is screwed to another piece *I* and closed by a bored leather gasket *K*. *I* contains another gasket *L* made of fat cotton-thread, which is compressed by means of the bored piece of brass *M* and the nut *N*. Through the whole passes a steel pin *O* of a little less than 4 mm. diameter, which fits exactly in the bore *P*. The connection between *O* and *P* is in such a manner that *O* needs not turn with *P*, but is drawn back, if *P* returns to its former position. The apparatus is filled by screwing *A* in

*F* with spring and ball in the required position and screwing on *E*, after this the opening *A* is filled with blood by means of a capillary tube and now *A* can be brought in every direction without the blood flowing out. The pin *O* should entirely be returned to its former position and *A* be screwed in *I*, after which when *P* is screwed on, the hemolysed blood flows off through *G*.

Howard determines the quantity of hemoglobin in blood by reading the refraction-index of hemolysed and unhemolysed blood with an Abbe refractometer. For the determination of the refraction of unhemolysed blood it is not necessary to free it from the erythrocytes. When a drop of blood is brought between the prisms of the refractometer and we wait some moments, we get a very distinct dividing line, which, according to the state of the blood, denotes the refraction of plasma or serum, let this be  $N_2$ . We can also easily determine the refraction of hemolysed blood, let this be  $N_1$ . It is clear that the difference between  $N_1$  and  $N_2$  will be the result of the solution of the contents of the erythrocytes in the plasma. This increase of the refraction index is chiefly the result of the hemoglobin going out. So there must be a somewhat simple relation between  $N_1$  and  $N_2$  and the quantity of hemoglobin. Now Howard found that  $N_1 - N_2 / 00183$  represented the quantity of hemoglobin in grams for every 100 c c blood. This scheme has not yet been fully worked out by Howard. He used for hemolysis either freezing and thawing repeatedly or saponine. The contents of the erythrocytes, other than hemoglobin, will probably exercise a different influence in different circumstances. This question Howard touches on, and I hope before long to be able to publish further particulars about it.

It may be worth mentioning that the specific weight of blood hemolysed in the manner described above (accurate to five places of decimals) does not differ from that of unhemolysed blood.

There is, finally, another use for this apparatus: we can emulsify with it two liquids that do not mix with one another, if first we make a rough emulsion by shaking and then force it through the press. In this way it is possible to prepare a fairly stable emulsion of paraffin oil in water.

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THE EFFECT OF CONCENTRATION OF THE RED  
BLOOD CORPUSCLES ON THE DISSOCIATION CURVE  
OF BLOOD. BY J. BARCROFT AND K. UYENO.

*(From the Physiological Laboratory, Cambridge.)*

THE following experiments form an extension of some observations which will be found in Appendix III of the "Report of the Peru High Altitude Expedition of 1921-2(1)." The suggestion underlying them was due to Dr C. D. Murray, who was working in this laboratory. The argument was as follows: (1) It is generally accepted, largely on the basis of Hamburger's work, that if the carbonic acid be withdrawn from shed blood (as by shaking) the chlorine ion will migrate from the interior of the corpuscles into the plasma, but the bulk of the metallic ion will remain in the interior of the corpuscle. (2) The blood may then be divided into two fractions by centrifuging, one relatively rich and the other relatively poor in corpuscles. (3) If the fraction rich in corpuscles be exposed in a saturator containing a given quantity of carbonic acid, this gas will be in part absorbed by the plasma and the chlorine ion will tend to return from the plasma to the interior of the corpuscles. (4) But inasmuch as the greater part of the chlorine ions were removed from the fraction rich in plasma and as there is an increased number of corpuscles relatively to the plasma, the quantity of chlorine which will migrate into each corpuscle is markedly less than that which the corpuscle originally contained at the  $\text{CO}_2$  tension under consideration. (5) This is another way of saying that the interior of the corpuscle is more alkaline than originally, when exposed to a given tension of carbonic acid. (6) In accordance with the work of Toyojiro Kato(2) the oxygen dissociation curve might be expected to be higher, i.e. the hæmoglobin would take up more oxygen at any given oxygen pressure between 0 and  $\infty$ , the other circumstances being the same.

The first object of the present research was to investigate the position of the dissociation curve under the conditions stated. If, however, the above argument is true other corollaries follow. (1) For the same reason that the dissociation curve of the concentrated fraction is raised, so in the fraction rich in plasma and poor in corpuscles, the dissociation

curve should be depressed as compared with that of normal blood (2) If instead of shaking out the  $\text{CO}_2$  before centrifuging, a high concentration of that gas was forced into the blood, one would expect the dissociation curves of the concentrated and reduced bloods to change place, that of the concentrated blood being depressed, whilst that of the fraction poor in corpuscles would be raised

Figs 1 and 2 given below show that these expectations were on the whole realised Little need be said about the experimental details which were quite straightforward The blood, in every case, was withdrawn from the finger by pricking with a needle, was defibrinated with a glass rod and was at once put on ice "Finger blood" which comes drop by drop cools much more rapidly than blood taken into a syringe—an important factor in view of the work of Evans(3) and others quoted by him The blood was used for experiment forthwith About ten minutes' shaking of the cold blood in a flask sufficed to remove a great portion of the carbonic acid After centrifuging 0.5 c.c. of plasma was removed in order to make certain by analysis that the  $\text{CO}_2$  had been sufficiently removed (or in the alternative case was present in large excess) A reading was taken of the blood which was to be analysed with a Haldane's hæmoglobinometer In the case of the fractions rich in corpuscles this reading was about 160 on the scale, whilst in the case of the fractions poor in corpuscles, the quantities of corpuscles and plasma were so regulated as to make the hæmoglobinometer reading about 60 When it was desired to centrifuge in the presence of a large quantity of carbonic acid, the blood was well shaken in a saturator in which the pressure of that gas was approximately 250 mm, from this saturator it was expelled under liquid paraffin into the tube of this centrifuge and a thick layer of that fluid remained above the blood during the fifteen minutes necessary for centrifuging

In the saturators which were used for the determination of the oxygen dissociation curve, a carbonic acid pressure of 25 mm was aimed at in every case and usually it was approximately obtained The data however have all been corrected by means of the Henderson(4)—Adair(5) line so that they correspond accurately to a tension of 25 mm of  $\text{CO}_2$

In Figs 1 and 2 the black line shows the dissociation curve of the subject's (Barcroft's) normal blood at 25 mm,  $\text{CO}_2$  pressure The curve is drawn from Hill's equation, the value of  $1/K$  being taken as 3600 Fig 1 deals with blood centrifuged after the  $\text{CO}_2$  had been largely shaken out, the amount remaining in being reduced to about 12 volumes p.c.

115  
DICAL CO<sub>2</sub>

Shortly after immersion a very obvious change takes place in the type of respiration; typically it changes from about 16 to over 200 respirations per minute. In the six experiments the lowest rate was 170 and the highest 250. This change is not associated with a corresponding change in the total ventilation per minute which so far from being increased twelve or fifteen fold was never increased three fold and on one occasion was not increased at all. In consequence the respiration became very shallow. In one experiment the tidal air was under 2 c.c. per minute, whilst in three others it was between 2 and 3 c.c. The result of this very shallow respiration was to lower the oxygen saturation in the arterial blood. The most striking case was one in which the saturation sank from 95 p.c. to 56 p.c., but as a rule it was between 80 and 90 p.c. during the periods of shallow respiration. Indeed it is remarkable that the blood was oxygenated to so high a degree as was the case considering that the tidal air amounted to but 2-3 c.c. This effect of shallow respiration is in agreement with the results obtained by Haldane and his collaborators(5).

The counting of the pulse during the periods of rapid respiration was impossible by palpation: it was achieved by piercing the chest walls with a needle, the point of which was placed on the surface of the ventricle; the heart beats were then counted on touching the protruding portion of the needle. The "minute volume" of blood passing round the body increased as the result of the warming of the animal as follows:

Minute-volume of blood in c.c.

Exp.	1	2	3	4	5	6	7	8	9
Before warming	352	310	168	223	223	227	259	402	283
During warming	539	415	218	234	363	280	311	482	349
Percentage increase	53	34	30	5	63	23	20	20	22

Systolic output in c.c.

Exp.	1	2	3	4	5	6	7	8	9
Before warming	1.4	1.9	1.1	1.2	1.2	1.3	1.0	2.0	1.5
During warming	2.9	1.7	1.8	1.3	1.8	1.3	1.7	2.5	1.6

The pulse rate increased somewhat, but not greatly, in five of the experiments; in one it became slightly slower, being at first abnormally rapid. In its comparative constancy the pulse differed markedly from the respiration. The systolic output was either approximately unchanged, or somewhat increased.

The following protocol of a single experiment may be taken as typical of all unless otherwise stated.

## Exp. 4. Urethane anaesthesia.

Condition of heat	Room temperature	In bath 43-41° C. for 30'
Rectal temperature ... ..	35° C.	39° C
Respiration rate per minute ...	16	240
Depth of respiration c.c. ...	16.7	2.7
Expired air c.c. per minute ...	277	640
O <sub>2</sub> p.c. in expired air ... ..	15.33	18.36
O <sub>2</sub> p.c. in inspired air ... ..	20.96	20.96
O <sub>2</sub> absorbed p.c. ... ..	5.63	2.60
O <sub>2</sub> absorbed in c.c. per minute	14.5	16.6
Saturation with oxygen p.c. :		
Arterial blood ... ..	99	92
Venous blood ... ..	57	50
Difference p.c. ... ..	42	42
Difference c.c. per 100 c.c. blood	6.5	7.1
Minute-volume of blood ...	223	234
Pulse rate ... ..	180	204
Systolic output c.c. ... ..	1.2	1.3

The question arises—To what must the increase of minute-volume be attributed? The following causes suggest themselves.

1. It has been shown that the warmed animal is suffering in some degree from anoxæmia. In the preceding table it will be seen that the percentage saturation of both the arterial and venous bloods drops when the temperature of the cat rises beyond a certain point. In connection with the arterial blood it must be borne in mind that even so small a drop in saturation means a large fall of the oxygen tension. Experiments on gassed animals quoted by Barcroft(3) suggest that increased minute-volume might be a compensation to anoxæmia of this type, but the experiments of Doi negative this view in anaesthetised cats(2), and the results of the Peru High Altitude expedition, 1921-2, point in the same direction in man(4).

2. It has been shown by Meakins and Davies(5) and by Barcroft and Nagahashi(6) that plunging the arm of man into warm water quickens the circulation so much as to make the blood in the superficial veins quite arterial in appearance. It will appear later that a similar effect may be produced in the cat. It seemed probable in Exps 1-6 that the minute-volume was increased merely by the augmentation of the skin circulation caused by the direct stimulus of the hot water. This point will be discussed later. Here, however, it may be said that a comparison of Exps. 1-6 with 7-9, and also 16 with 17, excludes the idea that stimulation of the skin by contact with hot water was the underlying cause of the increased minute-volume.

3. It will be noted that there is a considerable increase in the metabolism of the animal as shown by the oxygen intake; in the experiment



cited above it rose from 14.5 to 16.6 c.c. per minute, a rise of 15 p.c. This was a minimal figure, in one experiment it rose about 100 p.c. The cause of the rise is to be sought primarily in the rise of body temperature. The temperature of the cat under urethane was subnormal at the time that the first determination was made, whilst it always rose in the bath to a supernormal figure. The rise was usually about 4° C. except in Exp. 2, where it was only 1.5° C. In cases (two) where the increase of metabolism was extreme (doubled) an additional cause must be sought in some degree of muscular movement.

4. As has already been said, the respiratory movements are greatly augmented. Not only are they augmented in frequency but also in force. This is made manifest by the Müller's valves, in which the movements of the water are so great as to indicate quite unusual changes in intra-thoracic pressure. It might be expected that the increased pumping action of the chest would in itself cause as great a change in the minute-volume as was observed in several of the experiments.

Pending the results of an investigation at present being carried out by Hugget, it is not possible to apportion the extent to which the increased minute-volume is attributable to the increased metabolism and to more active working of the respiratory pump respectively.

The point raised under (2) requires fuller discussion. To investigate it I have made analyses of the blood flowing from the saphenous vein. This vein is mainly a cutaneous vein though it receives some blood from the muscles and bones of the foot. Its cutaneous area in the foot includes the area with sweat glands. In estimating the rate of blood flow from it, it is necessary to avoid any increase of pressure, as this would tend to divert some of the blood from the foot to the external saphenous vein by way of the anastomosis between these two veins. In the cats used I estimate the area of the skin supplied by the vein to be  $\frac{1}{18}$ – $\frac{1}{25}$ th of the whole skin area.

Exps. 10–12 show the relation of the changes in the oxygen content of the arterial blood, the blood in the saphenous vein and the blood in the right ventricle of the heart. Exp. 10 may be taken as an example, though the effect is more marked than sometimes occurs.

Oxygen saturation of blood.

Percentage saturation

	Percentage saturation			A - B	A - C
	A O <sub>2</sub> in arterial blood	B Saphenous vein	C Right ventricle		
Cat exposed to					
Room temperature	99	74	68	25	31
Bath 44° C. ...	90	82	43	8	47
Bath 15° C. ...	96	55	58	41	38

Starting from the condition of the cat at room temperature the saturation of the blood in the saphenous vein rises in the warm bath whilst that in the right ventricle falls, and the reverse changes take place in the cold bath. Were the conditions of metabolism constant this result would indicate a greater degree of vascular constriction in the animal generally than of dilatation in the skin, but as the conditions of metabolism are inconstant both in the body generally and the saphenous area in particular no such interpretation can be put on the figures.

In order further to explore the relative changes in the superficial area and in the body generally some fresh experiments were undertaken.

Exps 13-15 may be passed over rather lightly, in them the cat was wrapped in cotton-wool with a warm water bottle. In each case there was a considerable increase in the blood flow of the saphenous vein, though the temperature of the body rose but one degree. The increase was in Exp 13, 130 p.c., Exp 14, 64 p.c. and Exp 15, 146 p.c. The metabolism of the saphenous area was much increased. The data for Exp 14 are as follows:

Condition of animal	Blood flow per minute from saphenous vein	Oxygen consumption of saphenous area per minute	Temperature of cat
Room temperature	0.64 c.c.	0.14 c.c.	37° C
Warmed with bag and wool	1.05	0.50	38

The experiment is of some interest as showing a 3-4 fold increase of metabolism with an increased blood flow. This increase may be to some extent due to the actual change in temperature of the skin itself, but probably also was attributable to the activity of the sweat glands in the foot, in a later experiment we observed the appearance of sweat on the pads when the cat was warmed. Though of some interest in themselves Exps 13-15 did not yield the information desired, namely, whether the increased blood flow in the skin was of the same order as the total increase in the minute volume. Two other experiments were undertaken, 16 and 17, in which each quantity (namely (1) increased minute volume in the saphenous area, and (2) that in the body as a whole) was measured and compared. These experiments agreed in showing that the increased minute volume of the body generally is greater than that in the skin. In Exp 16 the increased minute volume of the blood from the heart was 107 c.c., that from the saphenous vein was 2.18 c.c. The skin area of the saphenous vein in this experiment was estimated as  $\frac{1}{18}$ th of that of the whole of the skin. On this basis the increased minute volume of the whole skin was 44.7 c.c., about  $\frac{3}{4}$ th of the increased minute volume of

the whole body. In Exp. 17 the estimated increase of the minute-volume of the skin was only about  $\frac{1}{8}$ th that of the whole body.

Exp.	16		17	
Body weight ...	2.0 kg.		3.3 kg.	
Condition ...	Room	41° C.	Room	41-40° C.
	temperature	bath for 30'	temperature	bath for 30'
Rectal temperature ...	36° C.	41° C.	36° C.	37.5° C.
Pulse rate per minute ...	165	252	174	200
Respiration rate per minute ...	28	240	34	246
Exp. air c.c. per minute ...	325	915	414	1111
O <sub>2</sub> p.c. in exp. air ...	15.77	16.25	15.36	16.59
O <sub>2</sub> p.c. saturation arterial ...	97	96	96	94
O <sub>2</sub> p.c. saturation venous ...	65	39	78	68
Minute volume, c.c. ...	355	462	689	1026
Depth of respiration ...	11.6	4.0	12.1	4.3
O <sub>2</sub> p.c. saturation saphenous	39	69	72	87
Blood flow c.c. per minute	518	3.0	91	3.0
Increased blood flow in saphenous area $\times 18$ in Exp. 16 and $\times 20$ in Exp. 17 for whole skin area ...	44.7 c.c.		40.2 c.c.	

A single experiment for which I have to thank Surgeon-Commander Farrley, R.N., Major Marshall, R.A.F., and Capt. Blackmore, R.A.M.C., served to establish for the cat the observation made by Richet(8) on the dog, that tachypnœa caused by warming the skin is not abolished by section of the vagi. The experiment shows that the tachypnœa is independent of influences passing to the brain from the lung, but it does not show whether the altered rhythm is due to warming of the centre as in the experiments of Goldstein and of Gad, who obtained tachypnœa by heating the blood in the carotids, or to raising the temperature of the skin as was supposed to be the case by Richet.

Two cats were anæsthetised with urethane, in one both vagi were cut, in the other they were left intact. Each cat was placed on a warm table and wrapped in cotton-wool, the rectal temperature was observed and the respirations counted. The cat in which the vagi were cut responded more rapidly to temperature but never reached so high a rate of respiration as that in which the vagi were uncut. No stress is laid on these differences. The following are the most salient of a large number of observations.

Rectal temperature	Respirations per minute	
	Cat A. Vagi cut	Cat B. Vagi intact
36° C.	25	40
39.3	240	150
40.4	235	390
42	—	400

These results do not of course prove that the essential rhythm of respiration is central, they could be accounted for on the assumption that the centre in the warmed animal responded more readily than normally to such afferent influences as remain after the vagi are cut.

The above experiment presents a striking contrast to the cases of rapid shallow respiration investigated by Dunn(7); in these the change in the type of respiration was caused by treatment of the lung with pulmonary irritant gases or by the production of emboli in the pulmonary vessels. In Dunn's cases the rapid type of respiration was abolished by section of the vagi and the essential cause was therefore presumably peripheral.

## CONCLUSIONS.

In the cat under urethane a rise of body temperature from about 36° C. to about 39° C. brings on the following phenomena: (1) very rapid and shallow respiration, (2) increased consumption of oxygen, (3) increased total ventilation, (4) fall in saturation of arterial blood (usually), (5) greater fall in saturation of mixed venous blood, (6) increase in "coefficient of utilisation," (7) increased minute-volume of blood for the body generally of the order of 20-30 p.c., (8) slight increase in systolic output (usually). In the saphenous area there is (9) increase in the saturation of the venous blood, (10) increase in the minute-volume frequently of the order of 600 p.c. On a very rough computation based on the saphenous area, the increased flow in the skin fails to account completely for the increased flow in the body as a whole. (11) The increase in rapidity of respiration is not abolished by section of both vagi.

The general increase in the blood flow is to be linked up with either or both of the following causes: (1) increased metabolism, (2) the increased action of the respiratory pump, but not merely with the effects of warm water on the skin or anoxæmia due to the shallow respiration.

In conclusion I should like to express my thanks to Prof. Langley for granting me the facilities of the laboratory and to Mr J. Barcroft for his advice and assistance during the course of the experiments. Dr Haldane kindly made some criticisms.

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# AN ANALYSIS OF THE PRODUCTION OF HEAT IN CERTAIN MUSCLES OF THE HEDGEHOG.

By W. HARTREE<sup>1</sup> AND R. J. S. McDOWALL.

*(From the Physiological Laboratory, Cambridge.)*

IN a previous paper(1) by one of us, it was shown that certain isolated muscles of the hedgehog were capable of surviving for a considerable time and of working at lower temperatures than that of the body; records were given proving that the processes of contraction and relaxation were much slower than in frog's muscle. It was suggested to us by Prof. A. V. Hill that an investigation of the heat-production in these muscles would be of value, partly because the hedgehog is a warm-blooded animal and all previous experiments on the heat-production of muscles have been made on cold-blooded animals, partly because the slowness of action of these muscles of the hedgehog would make it less difficult to analyse and to follow the true course of the heat-production in its initial stages. A series of experiments was made employing the usual arrangement of muscle chamber, thermopile and galvanometer(2).

The muscles used were (according to Huxley's(3) nomenclature) (A) the coccygeo-orbicularis, and (B) the humero-dorsalis. Preparation (A) has the advantage that it can be dissected out and the bone left attached to one end of each of the pair of muscles, and the bone can then be held in the clamp, in a manner similar to that in which the pelvic bone is held when using a pair of sartorius muscles. Preparation (B) was generally used as a single muscle turned round the end of the thermopile, so that it lay on both faces of the latter. The cervico-orbicularis was also tried and did not give good results.

A certain amount of damage was inevitably done in tying a thread directly on to the muscle substance, as had to be done at one end of each of the pair of muscles in case (A) and at both ends of the single muscle in case (B); during the tying a violent contraction was always produced. The damage so caused may account for the early failure of many of the muscles. It was usual to find that the muscle continued to give a mechanical response, on stimulation, for many hours after dissection; the tension and heat-production however, were often so small,

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and the stimulus required so great, that often a considerable portion of the heat observed was due to the direct heating effect of the stimulating current. This direct heating effect of the stimulus was always determined when a muscle was dead (on the supposition that the resistances, etc., were the same both before and after death) and allowed for, but when the allowance was large it naturally diminished the effective size and accuracy of the galvanometer deflection obtained, on which the analysis of the heat depends. The muscles used were generally four or five times the weight of a pair of frog's sartorii, but this has little effect on the size of the galvanometer deflection which more nearly represents a given change of temperature than the production of a given quantity of heat.

It was thought at first that the exposed portions of the muscle, especially those portions exposed to the thermopile itself, might be dying more rapidly than the internal portions of the muscle, and so might affect the apparent time course of the production of heat. It is obvious that if the muscle fibres directly in contact with the thermopile were dead and the fibres inside the muscle were still alive, the heat would always be delayed in its appearance at the thermopile. The records show however that a higher proportion of the initial heat than is usual with frog's muscle, is produced immediately on stimulation, so that no possible injury of the surface of the muscle by dissection or exposure can account for the actual phenomena observed, as they necessitate an effect in the opposite direction. It was found that the best results were obtained by keeping the muscle in ordinary mammalian Ringer's solution until the temperature in the chamber had practically settled down, in which case readings could be taken comparatively soon after the solution had been removed.

The size of the tension and heat readings with a given strength of stimulus varied considerably in different experiments, presumably in consequence of a difference in the condition of the muscles. In a few, fair sized readings were obtained with a strength of stimulus no greater than would have to be used to obtain similar readings with frog's muscle. Most of the experiments were carried out in air. Substitution of oxygen for air did not cause any marked difference, except in the recovery heat.

Various temperatures between 0° C. and 30° C. were tried. The temperature of the animal during life is high; in the abdomen soon after death it was 33° C. and we had expected that the muscle would survive better at 30° C. than at a lower temperature. On the other hand it seemed important to use as low a temperature as possible, so as to slow down the course of the initial heat production to enable its analysis to

be made the more accurately. We found, however, after many attempts, that good results could not be obtained either at  $30^{\circ}\text{C.}$  or at  $0^{\circ}\text{C.}$  In every case in which the muscle survived long enough to give any readings at all, the tension or heat in the first observations was small, even with a large stimulus. In such cases reliable heat observations were out of the question, although tension records could still be made. We cannot explain the failure at  $30^{\circ}\text{C.}$ , though the failure at  $0^{\circ}\text{C.}$  is perhaps hardly surprising in the case of a warm-blooded animal. Experiments at  $20^{\circ}$ ,  $15^{\circ}$  and  $10^{\circ}\text{C.}$  were fairly successful, and the results are given in the abstract below. Since, however, the processes in hedgehog's muscle at  $10^{\circ}\text{C.}$  are no slower (as one of us has since ascertained) than those in toad's muscle at  $0^{\circ}\text{C.}$ , one of the reasons for the present investigation, namely that of slowing down the process taking place in the muscle so as to follow the course of the initial heat-production, is not of such importance as we thought at first. The action of toad's muscle is considerably slower than that of frog's muscle so that a considerable increase of accuracy in the analysis of the heat will be possible with toad's muscle, which survives well at  $0^{\circ}\text{C.}$  We consider, however, that the results we have obtained with hedgehog's muscle, do give decisive evidence on the other and equally important point, namely the similarity of the process taking place during the contraction of muscles of very different animals. Further, this is the first time that an investigation of the heat-production of isolated mammalian cross-striated muscle has been made.

*Results.* In each case only a single shock or a short tetanus of not longer than 0.25 sec. (at 90 periods per sec.) was given. It was found that there was always a large outburst of heat immediately after the stimulus, which was rarely less than 60 p.c., and often as large as 70 p.c. of the total initial heat-production. Following this initial outburst was an interval, during which the tension of the muscle rises, and in which the heat-production is very small or nothing, the duration of this part being longer at a lower temperature. Following this there is a considerable evolution of heat, more or less following the process of relaxation, amounting altogether to some 30 to 40 p.c. of the total initial heat. The time relations of this heat-production associated with relaxation are shown in Fig. 1. There is no doubt whatever of its existence, or of the general type of curve governing its appearance. The rate of heat-production after the interval rises so quickly that it is impossible to assert that it does not start at its maximum value. The maximum rate of heat-production during relaxation is distinctly higher at a higher temperature,

and the rate of heat-production falls off much more rapidly at high temperatures as is shown in the following table.

Temperature in degrees C.	Maximum rate of subsequent H P. in terms of total initial heat per second	Approximate time to maximum rate, in secs.	Approximate time of rate falling from maximum to $\frac{1}{4}$ maximum in secs
10	.12	$1\frac{1}{2}$	3
15	.20	$1\frac{1}{4}$	$1\frac{1}{2}$
20	.30	1	$\frac{1}{4}$

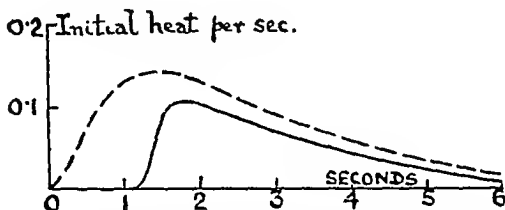


Fig 1. The tension (brokeo curve) and heat rate (full curve) for single shock at  $10^{\circ}\text{C}$ . The initial outburst of heat occurs very suddenly and is not shown in the figure, it represents about 0.07 of the total initial heat. Following this initial outburst is an interval of rather more than a second, after which the heat rate rises again, the total area of the heat rate curve, if continued till it touches the base line, being about 0.33 of the total initial heat.

The figure gives a good idea of the slowness at  $10^{\circ}\text{C}$ . of the process involved. Unfortunately tension records were not taken in every case. Two experiments at  $10^{\circ}\text{C}$ . gave a curve similar to that shown, but it must be observed that in most of the other cases in which tension records were taken, the tension fell off in relaxation rather more rapidly compared with the fall of the rate of heat-production. It will be seen therefore that, in general character, the hedgehog's muscle behaves in a manner exactly similar to the muscle of the frog. There is a large initial outburst of heat followed by an interval during which the tension rises, or continues to rise, and then there is a considerable heat-production during relaxation amounting to 30 or 40 p.c. of the total initial heat. This relaxation heat represents presumably, at any rate in part, the potential energy dissipated in the muscle during the process of relaxation.

It was possible to carry the analysis of the heat-production beyond the initial stages, and to determine the heat-production during the longer intervals in which recovery occurs. Some difficulty was found in obtaining reliable results, on account of the time which had to be given



after the Ringer's solution was removed and before the galvanometer was steady enough to take long records. Frequently the muscle had failed before sufficiently reliable records were possible. Three experiments however at 20° C. in air (from which the oxygen had been partly used during earlier contractions) gave very similar results, the total delayed heat being between 0.6 and 0.7 of the total initial heat, with a maximum relative rate of .004 per sec., at about 50 seconds after a tetanus of 0.25 sec. One experiment only was successful in oxygen, which at 10° C. gave a total delayed heat about 1.5 times the initial heat, with a maximum relative rate of .0025 per sec. at about 120 secs. after a tetanus of 0.2 sec. In this latter case a fairly large allowance had to be made for the heat appearing after 5 minutes, recovery being very slow on account of the lower temperature. It is seen however that in general the delayed heat during the recovery phase is similar, both in amount and in character, to that occurring under the same conditions in a frog's muscle.

#### SUMMARY.

An analysis of the heat-production during and after the contraction of the slow-moving isolated muscles of the hedgehog has shown that, qualitatively and quantitatively, the same phenomena occur as in frog's muscle. There is an initial outburst of heat on stimulation, continued if the stimulus be prolonged: an interval at the cessation of stimulation, followed by a large and characteristic outburst of heat during relaxation: and finally there is a slow, prolonged and considerable evolution of heat during recovery.

The expenses of this research have been borne in part by a grant from the Royal Society.

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## THE PART PLAYED BY THE DUCTS IN THE PANCREATIC SECRETION. BY L K KOROVITSKY

(From the Physiological Laboratory, University of Odessa)

THE first object of this work was to compare the secretory innervation of the pancreatic gland in the dog with that in the cat, an animal hitherto very little used for experiments on pancreatic secretion. In view of the striking difference between these two animals in respect of the sympathetic secretory fibres of the submaxillary gland, the comparison of the pancreatic innervation seemed to be interesting. Previous observers have shown that the stimulation of the vagus does not cause pancreatic secretion in all animals, in the rabbit for instance the stimulation of the vagus was found to have no effect(1), pilocarpine does not cause a flow of pancreatic juice in the pigeon(2), on the other hand the definite secretory effect of the vagus in the case of the dog is well established. In the cat the innervation of the pancreatic gland has never been studied.

*Method* The cats received their last meal 12 hours before the experiment. The animal was anaesthetized with an A.C.E. mixture, both vagi were then cut in the neck, a cannula tied in the trachea, the decerebration performed and artificial respiration started. Cannulae were also introduced into the duodenum and into the pancreatic duct. A longitudinal incision was made through the pylorus, the mucous membrane of the latter was separated from the muscular coat, a strong ligature passed between the two and tied. In this way the stomach was completely separated from the duodenum, with the least injury to the nerves which run in the muscle coat of the pylorus. The pancreatic cannula was connected with a narrow glass tubing fixed to a millimetre scale. The secretion of the pancreatic juice is given in all experiments in terms of millimetres of this glass tubing per minute.

*The action of the vagus nerves on the pancreatic secretion* Pavlov(3) has shown that in the spinal dog the stimulation of the vagi in the neck or below the heart in the chest causes a marked secretion of the pancreatic juice and that this juice is different in properties from the juice obtained with the aid of hydrochloric acid or secretin. The first question raised was whether the vagus has a similar action in the cat.

20 c.c. of 0.4 p.c. HCl were injected in the duodenum of the cat at the beginning of each experiment; this was done in order to verify the correct position of the pancreatic cannula, and the absence of kinks of the duct. When the secretion had completely stopped the vagus was stimulated either in the neck or in the chest above the diaphragm; a faradic current rhythmically broken by a beating metronome was used for the stimulation. Nine experiments were performed and in none was there observed any secretion of the pancreatic juice. Increase in the strength of the stimulus did not alter the fact. It seemed from these experiments that in the cat the secretory fibres are either absent or that their action is entirely different from that in the dog.

Pavlov(3), Popielsky(4), Mett(5) and Koudrevetsky(6) state that in the dog the vagus contains both secretory and inhibitory fibres. According to these observers the pancreatic secretion begins only after a long latent period and can be temporarily stopped by successive stimulation of the vagus. Their explanation of the long latent period is that the inhibitory fibres of the vagus have first to be tired out by a prolonged or repeated stimulation, and that then only can the secretory fibres produce an obvious effect. The absence of the secretion in the cat might have been due to the stronger action of the inhibitory fibres of the vagi over that of the secretory. It was hoped that if this was the real cause of the negative results, a prolonged stimulation of the vagi would tire out the inhibitory fibres and a secretion would be obtained. But stimulations which were continued for over 2-3 hours proved as ineffective as the short ones. Section of the vagus five days before the experiment gave no sign of selective degeneration of inhibitory fibres, for no secretion could be obtained by vagus stimulation.

*The action of pilocarpine. The augmented secretion.* Intravenous injection of pilocarpine causes in the dog a flow of pancreatic juice, but in the cat it has either only a very small effect or generally none at all. Repeated injections of 1-2 mgms. of pilocarpine were tried in 15 experiments without any definite effect on the pancreatic secretion. One rather curious fact was, however, noticed. As previously stated 20 c.c. or 0.4 p.c. HCl were injected into the duodenum at the beginning of each experiment; the secretion was measured and pilocarpine was injected only after its complete cessation. The absence of secretion after an injection of pilocarpine was unexpected and in order to make sure that this is not due to an occlusion of the duct a further 20 c.c. of the acid were injected into the duodenum. The second injection of the acid caused a pancreatic secretion very much larger than the first. This

experiment was repeated on many animals and it was invariably found that an injection of acid into the duodenum causes a larger and some times an enormous secretion after a preliminary intravenous injection of pilocarpine or after a prolonged stimulation of the vagus

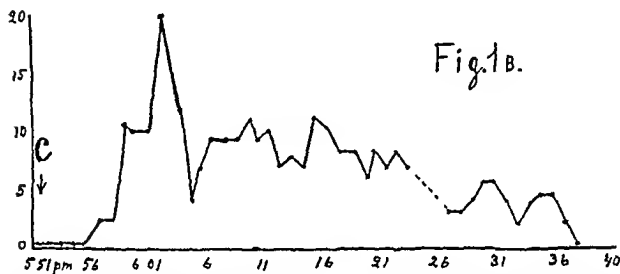
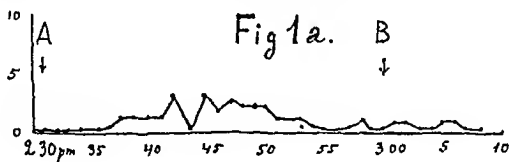


Fig 1a and b Secretion in millimetres of the glass tubing recorded each two mins At 4 20 c.c of 0.4 p.c HCl were injected in the duodenum—total secretion 20 mm From B to C=2 hours 40 mins rhythmic stimulation of the vagus in the neck—no secretion At C a second injection of the same amount of acid in the duodenum—total secretion 278 mm

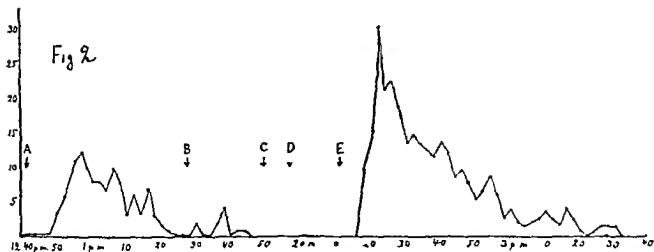


Fig 2 Secretion recorded every 2 mins At 4 20 c.c of 0.4 p.c HCl was injected in the duodenum—total secretion 96 mm at B C and D—intravenous injections of 1-2 mgms of pilocarpine, at E a second injection of the acid—total secretion 274 mm

and squeezing out of the juice which still remained in the gland. No further secretion was observed during the whole of the stimulation.

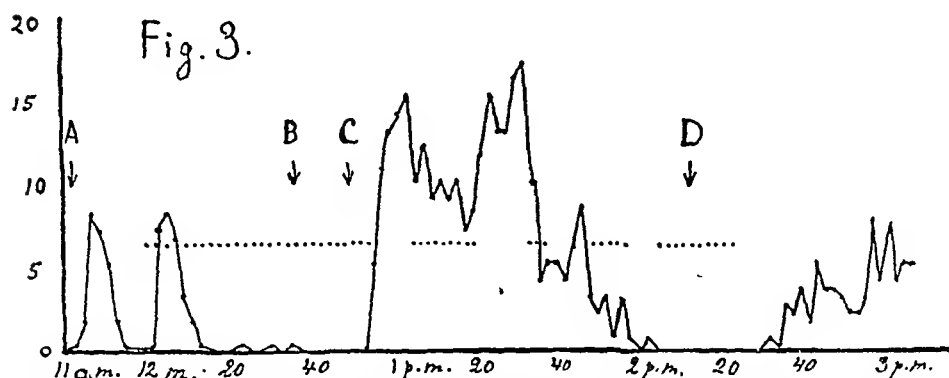


Fig. 3. Secretion recorded each 2 mins. At A, B, C and D injections of 20 c.c. of 0.4 p.c. HCl in the duodenum. The dotted line across the figure shows the time when the vagus nerve was stimulated. The nerve was stimulated in the neck, by a rhythmically broken Faradic current (coil 15-11.5 cm.), three minutes on, one minute off.

A second injection of the same amount of acid was made during the stimulation of the vagus; it did not cause a secretion either. Evidently the ducts were constricted to such an extent that the juice had no chance of escaping into the cannula. The stimulation of the vagus was then stopped and a third injection of the acid made. It now caused a free secretion of the juice in a much larger amount than before. Stimulation of the vagus produced each time a diminution of the outflow of the juice. A fourth injection of acid was made during a stimulation of the vagus. It did not cause secretion, but 20 minutes after the injection when the stimulation was stopped, the juice began to flow. Apparently the constriction of the duct had disappeared and the accumulated juice had now a free outflow.

*Experiments with perfused pancreatic ducts.* The animals were prepared in the same way as described above. Two cannulas were tied in the pancreatic duct instead of one. One cannula was introduced in the usual place near the entry of the duct in the duodenum. The other cannula was introduced near the tail (splenic) end of the gland; a small portion of the duct being dissected with a blunt instrument; in the first experiments the duct was generally injected with Indian ink to make it more visible, this was, however, abandoned in further experiments. The "tail" cannula was then connected with a Marriotte's flask filled with a 0.3 p.c. solution of  $\text{NaHCO}_3$ , with some addition of gum-arabic; a water-manometer was connected by means of a T-piece

to record the pressure; the perfusion fluid was warmed to body temperature. The outflow of the fluid from the pancreatic duct was recorded in the same way as the pancreatic secretion in the previous experiments.

In a dead animal the rate of the perfusion through the duct is very uniform and depends only on the pressure of the perfusion fluid. In an animal which is alive the ducts have a certain changeable tone of their own and the perfusion is not so regular. Stimulations of the vagus, intravenous injections of pilocarpine (1-2 mgs.) or small additions of pilocarpine to the perfusion fluid invariably cause a strong spasmodic constriction of the ducts as judged by the diminution or complete arrest of the outflow. At a pressure of 2-6 cm. of the perfusion fluid the constriction is strong enough to cause an arrest of the flow; at a pressure of 8-10 cm. there is only a more or less definite slowing. On some occasions, however, after repeated injections of pilocarpine the constriction of the ducts is so strong that the pressure has to be raised to 16 cm. to get a flow. Large doses of atropine paralyse the constriction mechanism of the ducts; the ducts dilate and the flow of the perfusion fluid increases. Intravenous injections of large doses of atropine (5-10 mgs.) or addition of atropine to the perfusion fluid abolishes also the constriction effect of pilocarpine (Figs. 4 and 5).

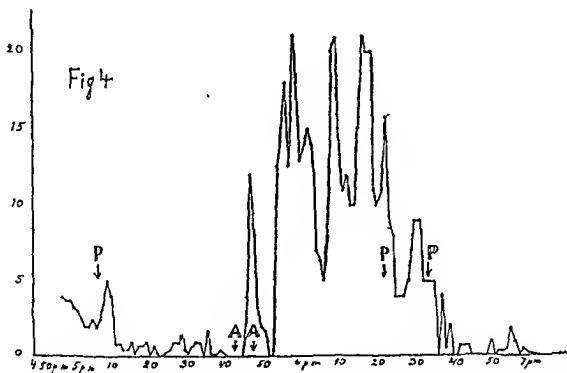


Fig. 4. Perfusion of the pancreatic ducts. The pressure of the perfusion fluid = 6 cm. Outflow records every minute. Intravenous injections of 1 mg. of pilocarpine were made at places marked P. Intravenous injections of 10 mgs. of atropine—at places marked A.

# ON THE CHANGE IN THE NATURE OF THE BLOOD SUGAR OF DIABETICS CAUSED BY INSULIN.

By W. DEVEREUX FORREST, M.D., W. SMITH

AND L. B. WINTER.

*(From the Biochemical Laboratory, Cambridge, the Department of Physiology, University of Durham, and the Royal Victoria Infirmary, Newcastle-on-Tyne.)*

IN a recent paper(1) it was shown that the normal blood sugar in man and animals has a lower rotatory power than would be given by the  $\alpha$ - $\beta$  equilibrium form of glucose as deduced from the copper reduction value. The sugar gives an osazone with the same crystalline form and melting point as that of glucosazone. The instability of the sugar is shown by its transient rotatory power, the curve of the polarimeter readings reaching the copper reduction value in three to four days in acid solution. The sugar at first decolourises potassium permanganate more rapidly than a solution of  $\alpha$ - $\beta$  glucose in similar concentration. This distinction no longer obtains when the polarimeter reading corresponds with that of  $\alpha$ - $\beta$  glucose. These facts, in conjunction with the work of Hewitt and Pryde(2) on sugar solutions introduced into the intestine, suggest that normal blood sugar is  $\gamma$  glucose. That ingested glucose or fructose is rapidly converted into normal blood sugar was shown by feeding experiments on normal persons. After 100 to 150 g. of glucose or fructose no alteration in the nature of the blood sugar could be detected.

In cases of diabetes mellitus it was shown that this sugar is not present in amounts capable of detection by the method employed. The polarimeter reading in these cases is initially greater than the copper reduction value. This suggests that besides  $\alpha$ - $\beta$  glucose, disaccharides, or other substances with a polarimeter copper reduction ratio greater than that of  $\alpha$ - $\beta$  glucose are present in the blood of diabetic persons. It was suggested that  $\alpha$ - $\beta$  glucose cannot be directly stored or utilised, but that an enzyme is responsible for the conversion of  $\alpha$ - $\beta$  glucose into  $\gamma$  glucose. The absence or inactivation of this enzyme was suggested as the direct cause of diabetes mellitus.

Macleod and his co-workers(3) have recently shown that injection of "insulin" into depancreatized dogs causes a decrease in the blood

sugar. Determinations of the respiratory quotient showed that sugar was being utilised as long as the administration of insulin was maintained. Similar results were obtained by the injection of insulin into persons suffering from diabetes mellitus. It was suggested that the effect of the insulin is to activate the enzyme responsible for the conversion of  $\alpha$ - $\beta$  glucoside  $\rightarrow$   $\gamma$  glucose, and that if this view is correct, examination of the blood sugar of diabetics after injection of insulin should show that the nature of the sugar approximates to that of normal persons<sup>(1)</sup>.

Some experiments which two of us<sup>(5)</sup> have performed with tissue extracts and glucose or fructose *in vitro* indicate that there are two factors concerned in the formation of normal blood sugar. These factors appear to be situated in the liver and pancreas. Solutions of glucose or fructose when incubated at 37° C. with very small amounts of insulin and liver extract in a jacketed polarimeter tube have their rotations altered in a downward and upward direction respectively. The copper reducing power of the sugar solution at the beginning and end of the reaction was unaltered. The reaction is therefore different from that described by Levene and Mayer<sup>(6)</sup> in connection with muscle and pancreas extracts, in which the copper reducing power was lowered. Boiled liver is inactive, whereas insulin is thermostable in this respect. Neither factor alone is capable of causing an appreciable alteration in the rotatory power of the sugar.

While it is probable that depancreatized animals will always be benefited by injections of insulin, in true diabetes, as in man, it is possible that the liver or pancreas, or both, may be impaired. The possible bearing of these facts will be discussed later.

On the view that sugar can only be utilised after conversion into a reactive form, presumably small amounts of  $\gamma$  glucose must exist in the blood of diabetics. This could not be demonstrated owing to the large amount of dextro-rotatory sugars present. The lowering of the blood sugar of diabetics caused by injections of insulin is probably due to a change in the equilibrium between  $\alpha$ - $\beta$  and  $\gamma$  glucose in the blood tending to increased formation of glucose which can then be stored or utilised. We have made some observations to determine how far this reactive form of glucose can be detected in diabetic blood after injections of insulin.

*Preparation.* Insulin was prepared by Collip's<sup>(7)</sup> method (we wish to thank Drs H. H. Dale and H. W. Dudley for permission to use some improvements in the method which they have recently devised). Owing to the difficulty of obtaining sufficient pancreas of any one animal, those



previously found, the curve of polarimeter readings in the cases before insulin tended, if anything, to fall: after insulin injections the curve rose definitely in three cases.

The amount of normal blood sugar was not so great as to show a decrease in the rate of decolourisation of potassium permanganate day by day. A slight difference in the rate of decolourisation by the final filtrate as compared with that brought about by a glucose solution of equal concentration is of no importance, since it is well known that salts, especially phosphates, accelerate the oxidation of glucose. It was owing to the difference in the rate of decolourisation day by day that it was suggested that the normal blood sugar is of a reactive type. It is only when a considerable amount of sugar of a reactive nature is present, as in normal persons, that this difference can be reliably demonstrated. It is probable that after a longer course of insulin injections the ratio polarimeter to copper reduction would approach the normal: our object has merely been to show that even after two or three injections of insulin the nature of the blood sugar has undergone a change. The differences in the curves of polarimeter readings before and after insulin may be seen in Fig. 1. Blood sugar curves after injection of insulin are shown in Fig. 2. After the administration of insulin the glycosuria disappeared and did not return for some hours.

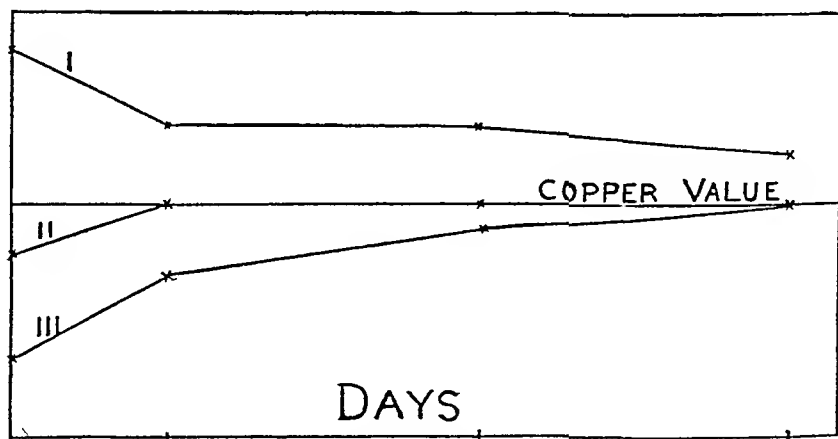


Fig. 1. Curves of polarimeter readings on successive days. I. Case 1. Before insulin. II. Case 1. After insulin. III. L. B. W. (normal). (The curves are scaled to a common copper value.)

On some of these cases a general  
been carried out. On admission fats

of diet had  
ate and

protein reduced to 10 grams of each per day; this was continued until the patient was sugar free, usually four days after admission. The diet

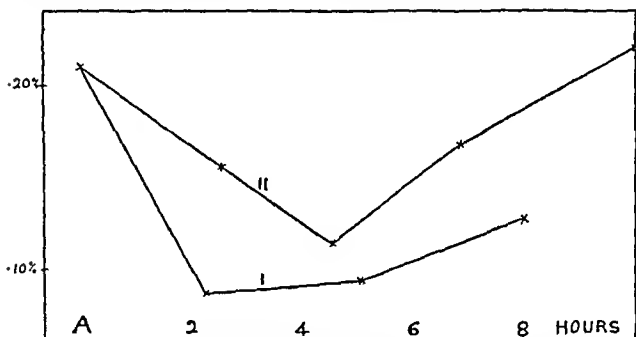


Fig. 2. Curves of blood sugar values after insulin. Insulin was given at A. I. Case 3. II. Case 6 (see Appendix).

was then gradually built up, still containing very little fat, until the tolerance limit was reached, as evidenced by the excretion of sugar. The diet was then again reduced until the patient became sugar free, the fat was then increased until the requisite calorie value was reached. In a previous paper(1) the samples of blood obtained from diabetic persons were all from severe cases before treatment. In the present work our results would tend to show that treatment by diet influences only the quantity and not the nature of the blood sugar. (Details are given in the Appendix.)

### Discussion.

Since insulin reduces the blood sugar of normal animals it seems feasible to conclude that the normal blood sugar is a mixture of  $\alpha$ - $\beta$  and  $\gamma$  glucose in a certain equilibrium. It has been shown that insulin and liver extract *in vitro* cause an alteration of the rotatory powers of glucose and fructose without a corresponding alteration of the copper reducing power. A possible explanation of the action of insulin is that it causes a shifting of the equilibrium in the direction of increased  $\gamma$  glucose formation. The increased amount of  $\gamma$  glucose available is then stored or utilised. The increased utilisation of sugar by the diabetic after injection of insulin may be put down to the increased formation of  $\gamma$  glucose. The difference in the ratio polarimeter to copper reduction of the sugars of

diabetic blood which we have found after insulin is in support of this view.

The severity of diabetes is probably inversely proportional to the amount of  $\gamma$  glucose which the body is capable of forming. The failure to form the normal amount of glycogen in the liver, coupled with the inability to burn fat may be due to the lack of adequate supplies of a reactive type of sugar, and consequent failure to accomplish the "glucosidic union" of Ringer<sup>(10)</sup>.

When excessive amounts of insulin are administered the lowering of the blood sugar may take place to an excessive degree. One case (No. 6) which received insulin in the evening, and on the following morning at 8 a.m., showed symptoms of a reaction: at 11 a.m. the patient complained of faintness and tingling in the fingers. Sugar was at once administered by the mouth, and relief was almost immediately experienced.

Another case (No. 5) felt no benefit from the insulin administered, the third injection given without food only reduced the blood sugar to .112 p.c. The curve of polarimeter readings of the sample obtained after insulin remained throughout above the copper value. The blood sugar before insulin was found to be abnormally high by both methods. It is possible that there may have been some derangement of the liver mechanism with the result that in spite of excess of insulin in the blood an appreciable amount of  $\gamma$  glucose was unable to be formed. Whether a definite improvement would result from continued treatment with insulin we were unable to determine, but this case definitely showed that impairment of some other function was associated with partial lack of the pancreatic factor.

That a continual supply of insulin is necessary for the working of the enzyme is shown by the fact that the blood sugar invariably rose between the insulin injections. When the effect of the insulin wears off,  $\gamma$  glucose which can be readily stored or utilised, is no longer formed in appreciable quantity and the bulk of the sugar circulating in the blood can now no longer be removed, with the result that the quantity rises up to the leak point of the kidneys.

We are much indebted to Drs T. Beattie, Horsley Drummond, W. E. Hume, and A. Parkin for permission to make use of their cases. We wish to express our deep gratitude to Mr W. P. T. Watts, M.B., for performing some of the estimations by Maclean's method. Without his assistance this work could not have been efficiently carried out. We

offer our grateful thanks to Professors F G Hopkins and D Burns for the interest they have taken in this work, and for facilities placed at our disposal

The expenses of this research were in part borne by grants made by the Scientific and Industrial Research Board (to W S) and by the Medical Research Council (to L B W)

### SUMMARY

1 Previous observations on diabetic cases are confirmed. The blood sugar of diabetics differs from that of normal persons as regards the ratio copper reducing polarimetric value

2 In diabetics the decreased amount of blood sugar caused by injections of insulin contains a greater proportion of normal blood sugar as shown by the alteration in the ratio copper reducing polarimetric value

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- (5) Winter and Smith *Proc Physiol Soc* p xxvi *This Journ* 57 1922
- (6) Levene and Mayer *Journ Biol Chem* 9 p 97 1911
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- (9) Folin and Wu *Journ Biol Chem* 38 p 81 1919
- (10) Ringer *Ibid* 17 p 107 1914

## Appendix.

Case 1			Blood sugar p.c.	Sugar in urine p.c.
Jan. 30	10.30	55 c.c. blood	·242	—
Jan. 31	11.30		·194	2
	11.45	2½ c.c. insulin	—	—
	3.00		·081	nil
	5.20		·125	"
	8.00		·140	—
Feb. 1	8.00 a.m.		—	trace
	10.15		·118	—
	11.30	85 c.c. blood	—	—
	12.30		·112	nil
	4.00		·118	—
	6.45		·130	nil

	Polarimetric readings on successive days	Copper reducing value
	R.	R.
Before insulin	·15, ·12, ·11, ·10	·09
After insulin	·05, ·06, ·06	·06

Case 2			Blood sugar p.c.	Sugar in urine p.c.
Jan. 30	10.30	25 c.c. blood	·144	—
Jan. 31	11.30		·200	·17
	11.45	2½ c.c. insulin	—	—
	2.00		·150	·38
	5.30		·178	trace
	9.00		·162	—
Feb. 1	8.00 a.m.		—	·21
	10.30		·112	—
	1.00		·137	trace
	4.30		·088	nil
	7.00		·131	—
Feb. 2	8.00	2½ c.c. insulin	—	—
	10.30	50 c.c. blood	·094	nil
	1.10		·112	"
	4.00		·200	—
	5.00		—	·62
	8.00		—	1·1
Before insulin		·08, ·08, ·08, ·09	·05	
After insulin		·05, ·07, ·08, ·07	·05	

Case 3			Blood sugar p.c.	Sugar in urine p.c.
Jan. 31	10.30	50 c.c. blood	·275	—
Feb. 1	11.00		·210	—
	11.10	2½ c.c. insulin	—	·5
	1.15		·088	nil
	4.30		·094	"
	7.00		·125	—
Feb. 2	9.30		·131	nil
	10.00	2½ c.c. insulin	—	—
	12.10		·112	nil
	4.00		·159	"
	7.00		·163	"
Feb. 3	8.00	2½ c.c. insulin	—	—
	11.00	115 c.c. blood	·074	"
	2.30		·123	nil
	6.00		·150	"

	Polarimetric readings on successive days	Copper reducing value
	R.	R.
Before insulin	·15, ·15, ·14, ·13	·05
After insulin	·09, ·09, ·11, ·11	·10

Case 4			Blood sugar p.c.	Sugar in urine p.c.
Jan. 31	10.45	55 c.c. blood	·312	—
Feb. 1	10.15		·196	·72
	10.30	2½ c.c. insulin	—	—
	1.00		·081	nil
	4.00		·075	"
	6.30		·100	"
Feb. 2	8.00	2½ c.c. insulin	—	—
	10.10	92 c.c. blood	·062	—
	12.30		·131	—
	2.00		—	nil
	3.35		·125	—
	7.00		—	trace
Before insulin		·17, ·18, ·17, ·16	·14	
After insulin		·10, ·12, ·11, ·12	·12	

Case 5		Blood sugar p c	Sugar in urino p c
Feb 1	10 30 45 c c blood	437	2
Feb 2	9 30	312	2 5
	0 45 2½ c c insulin	—	—
	12 15	275	25
	4 30	181	80
	6 40	240	2 2
Feb 4	12 00	277	2
	12 30 2½ c c insulin	—	—
	2 45	143	1 6
	6 15	204	nil
Feb 5	7 00 2½ c c insulin	—	trace
	10 15 67 c c blood	112	nil
	2 30	144	"
	8 00	186	trace

Polarimetric  
readings on  
successive days

R

R

Before insulin 22, 21, 23, 22  
After insulin 07, 08, 07

10  
05

Case 6		Blood sugar p c	Sugar in urino p c
Jan 30	10 30	210	—
	10 40 2½ c c insulin	—	—
	12 50	158	—
	3 00	114	nil
	5 15	17	"
	8 55	221	—
Jan 31	11 10	202	57
	11 15 2½ c c insulin	—	—
	1 30	10	nil
	5 00	151	"
	8 00	175	—
Feb 2	8 00 p m	—	42
	2½ c c insulin	—	—
Feb 3	8 00 a m	—	3
	2½ c c insulin	—	—
	11 30 40 c c blood	059	—
	11 35 20 g cane sugar	—	—
	12 45 18 g carbo hydrate	—	nil
	2 15	120	—
	0 15	171	nil
Feb 4	11 30	184	—
Feb 5	10 30 70 c c blood	22	—

Polarimetric  
readings on  
successive days

R

R

Before insulin 12, 12 11, 11  
After insulin 04

10  
05

Case 7		Blood sugar p c	Polarimetric readings on successive days p c	Copper reducing value p c
40 c c blood		29	13, 11, 11, 10	06

TABLE I. Blood sugar in mg. per 100 c.c. after intravenous and subcutaneous injections of insulin.

No.	Initial blood-sugar		½ Hr.		1 Hr.		2 Hrs.		3 Hrs.	
	Intra-ven.	Sub-cut.	Intra-ven.	Sub-cut.	Intra-ven.	Sub-cut.	Intra-ven.	Sub-cut.	Intra-ven.	Sub-cut.
1, 1 a	122	121	42	51	41	16*	60	30	—	—
2, 2 a	133	120	43	71	18*	21*	—	12	—	—
3, 3 a	110	123	61	79	34	58	26*	38	—	40
4, 4 a	113	111	69	84	64	84	48	70	53	65
5, 5 a	175	185	100	112	82	87	100	78	120	78
6, 6 a	130	190	60	80	60	80	79	87	126	82

\* Convulsions about this time..

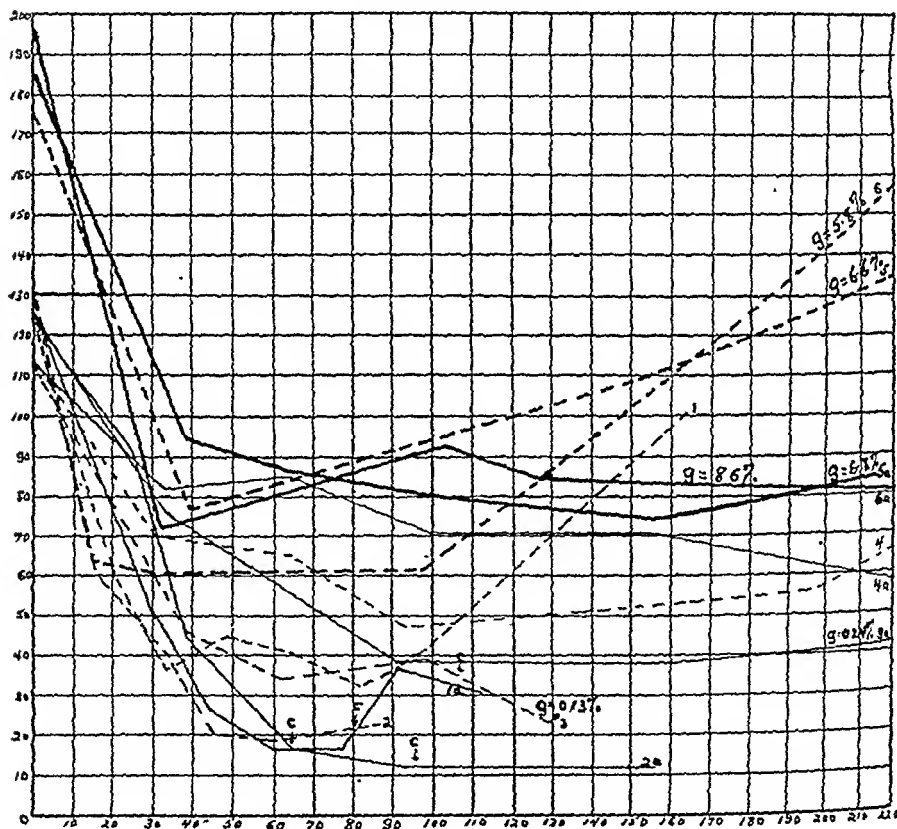


Fig. 1. See text.

With the exception of 6, 6a the initial percentages of blood sugar of each contrasted pair of animals, are sufficiently close together to permit of our taking the percentages found at the various periods following injection as safe indices of the effect of insulin. It will be seen that the fall in blood sugar in one half-hour was greater in the intravenously injected animals in all the experiments. After one hour the

values were markedly lower in both animals of three pairs (2, 3 and 5). In three pairs (3, 4 and 6) the intravenous was well below the subcutaneous, they were practically the same in one (5) and the subcutaneous was below the intravenous in another (1). In the subsequent behaviour the curves of each pair either ran very much alike (2, 2a, 3, 3a, 4, 4a, 6, 6a), or the curve for intravenous injection rose more rapidly (1, 1a, 5, 5a). The general conclusion which may be drawn is that there is no significant difference between the effects of subcutaneous and intravenous injection of insulin except that the fall in blood sugar during the first half-hour and the subsequent recovery may be somewhat more rapid following the latter method. The insulin used in these experiments was prepared from the pancreas of the skate, and it did not give any of the colour reactions for protein (biuret, Millon; xanthoproteic, Hopkins-Cole). It is possible that with less pure preparations containing traces of protein, the effects by subcutaneous injection might be more definitely prolonged than by intravenous.

*Comparison of the effects of subcutaneous injection of insulin into well-fed animals and into others in which the glycogen stores were reduced to a minimum by phloridzin and epinephrin.*

The results of three experiments of this category are given in Fig. 2, in which comparison is made between rabbits with practically no glycogen in the liver and others with abundance of this material. The dose of insulin per kilo body weight was the same in each pair of animals. It is plain that glycogen-rich animals withstand insulin much better than glycogen-poor, both when judged by the behaviour of the blood sugar curves and the incidence of convulsions.

The experiments are arranged in pairs in the order from above downwards of the strength of the dose of insulin given, as judged from the behaviour of the curves. In No. I, which shows the effects of a strong dose, convulsions (C) occurred in both animals, but these appeared an hour earlier in the starved than in the fed animal, and the blood sugar was decidedly below the .045 level when they were observed to occur. It was also noted in this and other experiments that the behaviour of the animals between the convulsive seizures was quite different according to whether they were fed or starved. The starved animals lay in a more or less unconscious condition and became progressively weaker and weaker with increasing coma, while the fed animals usually recovered remarkably and often were able to hop about in a tolerably normal manner between the convulsive seizures. The ultimate behaviour was



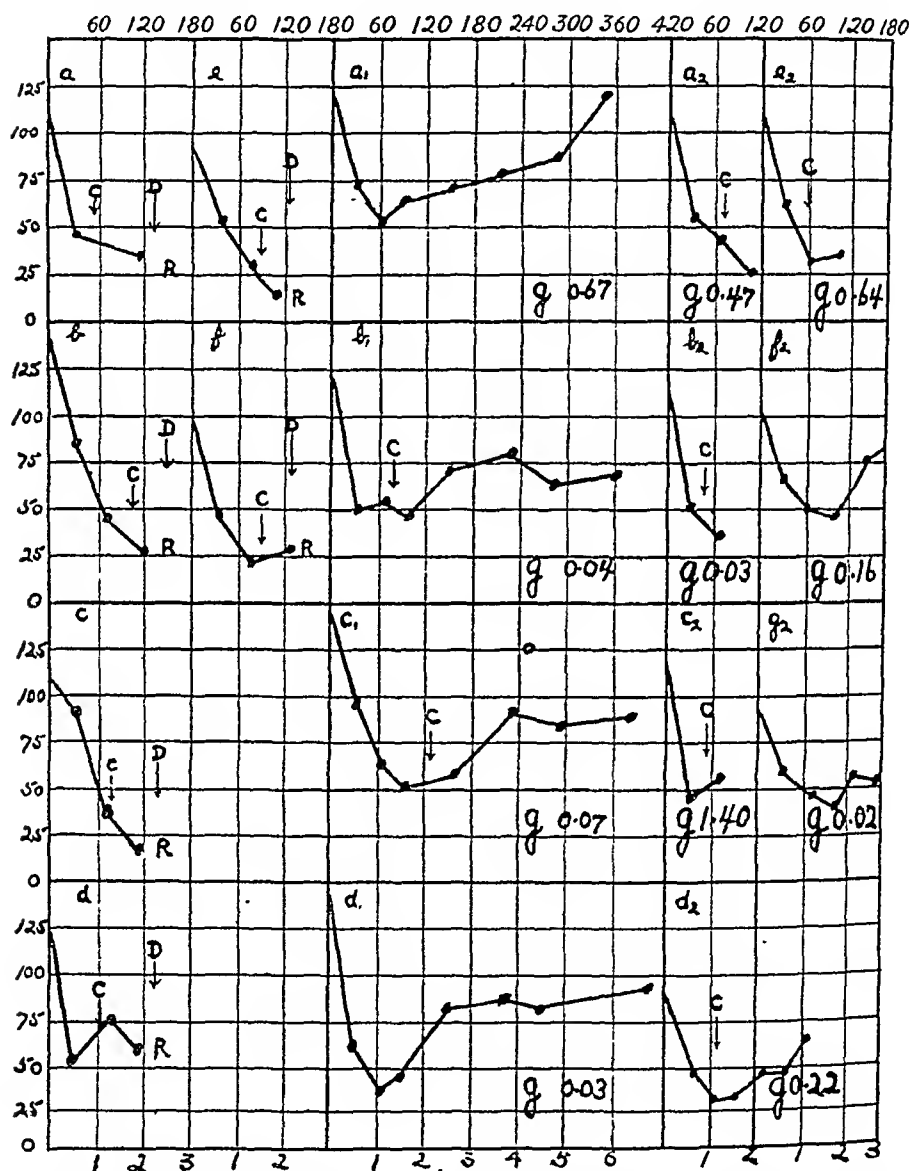
A. Starved 1 day  
0.6 c.c. InsulinB. Starved 1 day  
0.3 c.c. Insulin per kiloC. Starved 3 days plus  
Phloridzin and Adrenalin  
0.3 c.c. Insulin

Fig. 3. Blood sugar curves following the intravenous injection of insulin in amounts per kilo body weight indicated at the top of each group of observations A, B and C. *g* indicates the percentage of glycogen found present in the liver. Abscissae—hours after injection. Ordinates—mg. sugar per 100 c.c. blood.

The explanation may be that the cell changes which are immediately responsible for the occurrence of the convulsions and which are the result of a lowering of the amount of glucose dissolved in the tissue fluids (tension of glucose) have not had time to become established. As pointed out by Barany in a recent demonstration before the American Physiological Society the convulsions produced by insulin are an exaggerated form of those which are produced in normal animals by turning, and must therefore indicate changes in the labyrinthine mechanism. The exact nature of the change which is responsible for the irritation of this mechanism remains to be investigated; all we can state at present is that it is apparently related to the decrease in the percentage of glucose in the blood and it is possible that when this occurs rapidly, as in the experiments of the present group, it reaches a lower level before toxic substances, which are normally prevented from accumulating because of the presence of a certain intracellular "tension" of glucose, become developed. So far it has not been possible to detect any lesions in the central nervous system by post mortem examination. In every one of the animals of this group subcutaneous administration of 4 gm. glucose was followed by rapid recovery from convulsions, and the animals were returned to the pens.

In Group B the curves show the blood sugar in rabbits starved during one day and then injected intravenously with half the dose of insulin given those of Column I (viz. .3 c.e. per kg.). The difference between the results of A and B is mainly with regard to the incidence of convulsions. In only two cases of the latter group were these observed and they were of a mild form in contrast to those of Group A. The onset of these symptoms was also delayed. With this exception, however, there is no contrast in the immediate results following injection of .3 and of .6 c.c. of the same preparation of insulin, which would indicate that so far as the initial rate of decline of blood sugar is concerned a moderate dose has the same effect as an excessive one. The point of distinction between the moderate and excessive doses is more particularly with regard to the duration of their action. In the observations of Group A the sugar continued to decline up to the second hour after giving insulin, whereas in those of Group B it had usually begun to rise perceptibly by this time.

In Group C the effect of .3 c.c. insulin is shown in animals after more complete removal of the glycogen stores by the use of phloridzin and adrenalin. Compared with the results on animals that were merely starved (B) the main difference seems to be with regard to mortality

(not indicated in the curves). Five of the seven animals died, four of them within two hours of giving the insulin. The remaining two, however, showed complete recovery, the blood sugar returning to  $\cdot 143$  p.c. in 428 minutes in one of the animals, and to  $\cdot 086$  p.c. in 440 minutes in the other. Since there were only traces of glycogen in the livers of these animals the mobilized sugar was presumably derived from protein.

Two facts of importance in connection with the physiological assay of insulin are brought to light by these experiments, namely that neither the incidence of convulsions nor the percentage of blood sugar can be depended upon. With regard to the latter it is to be noted that although the same doses of insulin per kilo body weight were injected the extent of the falls in blood sugar were not uniform even when animals of exactly the same weight were used. In a general way it is true in each of the groups that the fall was less in the heavier animals, but there is obviously no definite relationship between body weight and susceptibility to insulin.

*Comparison of the initial fall and the rate of recovery of blood sugar after injecting insulin intravenously into starved and well-fed rabbits.*

The observations recorded in the preceding part of this paper led us to consider that it would be of value to repeat those of intravenous injection under more strictly controlled conditions and with more frequent measurement of blood sugar, partly in order to determine the degree of accuracy that might be attained in the physiological assay of insulin by measurements of the blood sugar and partly to throw some light on the nature of the factors involved in the decline and subsequent recovery in blood sugar. Three groups of experiments were performed and the results are shown in Figs. 4, 5 and 6. In each of the first two groups, varying doses of insulin were given to fed or moderately starved animals and in the third, equal doses were given to animals that were either fed on a large excess of carbohydrate or were rendered glycogen-free by injections of epinephrin. The insulin used for each group of experiments was different but it was the same for the different observations of each group.

The curves of Fig. 4 show the behaviour of the blood sugar in fifteen rabbits that were allowed food (oats and hay) up to the time insulin was injected in varying amounts which are indicated at the end of the curves by figure, giving the c.c. per kilo body injected in each case<sup>1</sup>.

<sup>1</sup> The letters after the figures are for reference to the notes and the figures along the curves give the body weights in kilograms.

The most outstanding feature of the results is that the fall in blood sugar is practically the same in all cases during the first 30 minutes of the

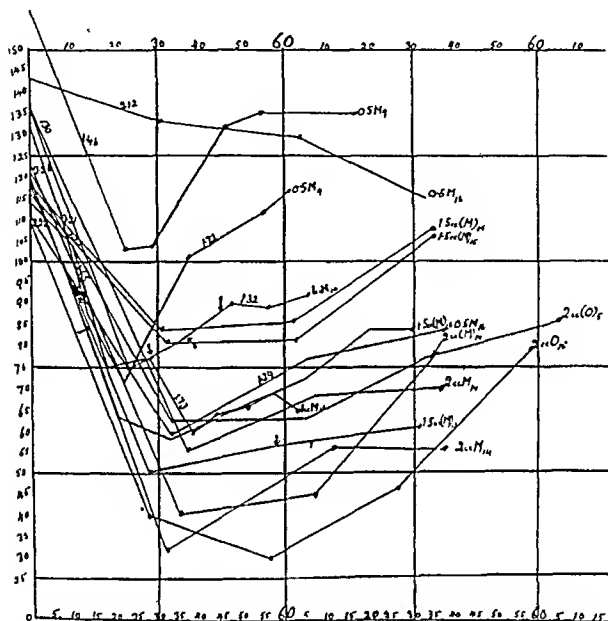


Fig 4 See text The weights of the animals are given by the figures part way along the curves Abscissae—minutes after injection of insulin Ordinates—mg sugar per 100 c.c. blood

observations. Indeed there are only three notable exceptions to this, viz  $M_{16}$  with a dose of .5 e.c. and the two observations of  $M_{15}$  with 1.5 c.e. each. It will further be seen that in most cases the lowest level was also attained at this time and that the recovery which followed was markedly irregular, so that in 60 and 90 minutes after the injections, the curves are spread over a much wider range than after 30 minutes. The parallelism of the curves for the first half-hour shows quite plainly that there can be no relation between the dose of insulin and the percentage of blood sugar at this stage, but if we assume that the rate of

recovery is dependent in part at least on the amount of insulin given, it becomes important to see whether the levels at 60 or 90 minutes might bear a satisfactory relation to the dosage. This is done in Table III, in which the values in 60 and 90 minutes are subtracted from those of the initial blood sugar and then calculated as a percentage of the latter. It is clear that no satisfactory relation exists between dosage of insulin and blood sugar in recently-fed animals.

Observations on animals starved for 24 hours are shown in Fig. 5.

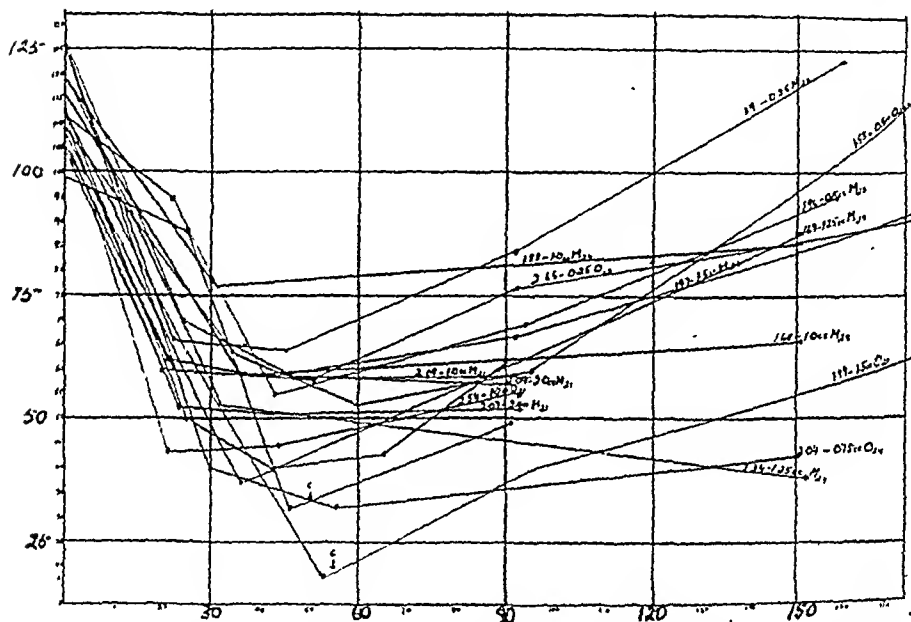


Fig. 5. Blood sugar curves in starved rabbits following the intravenous injection of the doses of insulin per kilo body weight indicated by the figures towards the end of each curve. The figures immediately in front of those of dosage give the weights of the animals. Abscissae—minutes after injection of insulin. Ordinates—mg. sugar per 100 c.c. blood.

The insulin was used in doses varying from .25 c.c. per kilo to 2 c.c., the last-mentioned dose being sufficient to cause convulsions in certain of the animals. It will be seen that the initial falls in blood sugar, as in the previous group, are similar for the varying doses administered the minima being reached a little later than in the fed animals. Comparison of the extent of the fall in the blood sugar is made in Table IV after 60 and 90 and 120 minutes, and it is evident that although the relation between dosage and the blood sugar values is somewhat closer than in the observations on fed animals it is not sufficiently close to be useful

TABLE III Effect on blood sugar at varying periods after varying doses of insulin Fed animals

After 60 minutes

Dose of insulin	1 cc/kg			1.5 cc/kg			2 cc/kg		
	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg
Normal BS	160	132	143	111	136	114	118	116	120
BS after 60'	137	116	130	074	096	067	085	082	057
Fall in 60'	023	016	013	037	046	047	033	034	063
Percentage fall	15.6	12.1	9.1	33.6	33.7	41.2	27.9	28.3	42.2
							70.5	47.8	64
									52.5
									58.7

After 90 minutes

Dose of insulin	1 cc/kg			1.5 cc/kg			2 cc/kg		
	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg
BS after 90'	?	117	082	?	?	105	102	061	084
Fall in 90'	—	—	026	029	—	013	014	059	037
Percentage fall	—	—	18.1	26.1	—	11	12.3	49.2	30.6
							54.1	37.2	41
									48.9
									53.8

TABLE IV Effect of blood sugar at varying periods after varying doses of insulin Animals starved 24 hours

After 60 minutes

Dose of insulin	1 cc/kg			1.5 cc/kg			2 cc/kg		
	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg
Normal BS	125	104	109	112	113	90	125	119	110
BS after 60'	070	062	53	02	43	37	48	49	62
Fall in 60'	55	42	56	50	70	62	60	72	48
Percentage fall	44.0	40.3	51.4	44.7	62	62.6	55.5	59.5	43.7
							57.2	57.2	57.2
									51.3

After 90 minutes

Dose of insulin	1 cc/kg			1.5 cc/kg			2 cc/kg		
	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg
BS after 90'	683	076	59	68	62	40	61	40	67
Fall in 90'	42	28	50	44	51	50	47	75	43
Percentage fall	33.6	26.9	45.8	39.3	45.2	50.5	43.5	62	39.2
									67
									53
									54
									56.5

After 120 minutes

Dose of insulin	1 cc/kg			1.5 cc/kg			2 cc/kg		
	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg	1 cc/kg	1.5 cc/kg	2 cc/kg
BS after 120'	100	081	76	80	—	—	74	42	75
Fall in 120'	25	23	33	32	—	—	34	79	35
Percentage fall	20	22.2	30.3	28.6	—	—	31.5	65.3	31.8
									38
									—
									—

as an accurate method of assay. It is also evident that there is no close relation between the size of the animal and the effect of insulin, when the dosage per kilo body weight is the same.

The most important feature which the curves of the two groups of observations bring to light is the difference in the rate of recovery of the blood sugar, this being decidedly slower in the starved animals than in the well-fed. Although a different preparation of insulin was used for the experiments of these two groups, there was very little difference in its strength.

Since the rate of recovery must depend partly on the strength of insulin and partly on the ability of the organism to compensate for the hypoglycæmia the observations shown in Fig. 6 were performed. In

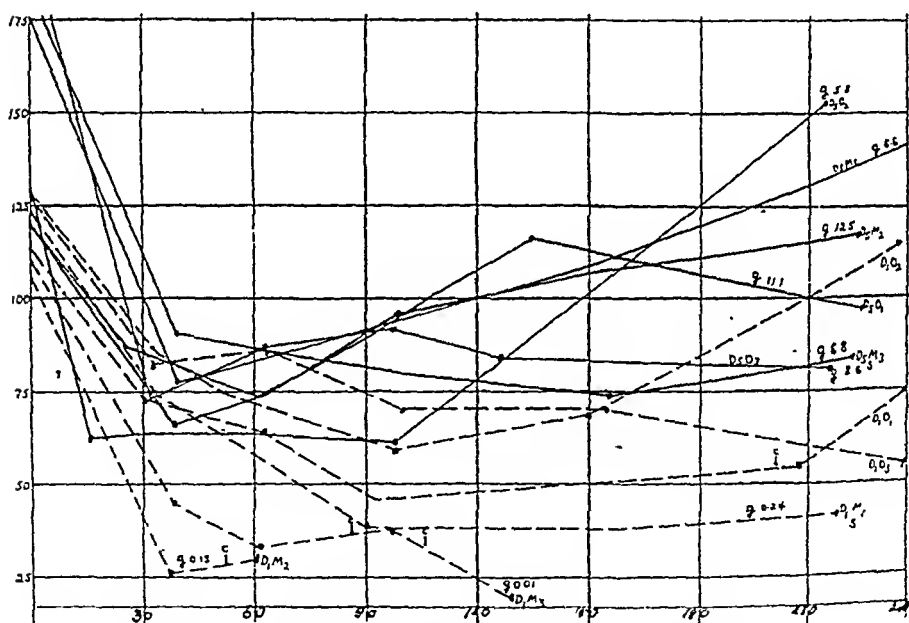


Fig. 6. See text. Abscissae—minutes after injection of insulin.  
Ordinates—mg. sugar per 100 c.c. blood.

these the same doses (per kilo body weight) of insulin were injected intravenously into rabbits some of which were fed with oats and sugar so as to cause much glycogen to be deposited and some were starved for three days and given epinephrin frequently so as to make them glycogen-free. The six curves in the continuous lines give the results in the glycogen-free rabbits and the six curves in the broken lines those in

glycogen rich rabbits<sup>1</sup> The percentages of glycogen are indicated on each curve It will be seen that the recovery in blood sugar set in after 30 minutes in four of the glycogen-rich animals, whereas in all of the glycogen free rabbits there was recovery in one case only and this was much delayed The failure of the non glycogenic animals to show recovery within the time of the observation indicates probably that a much larger dose of insulin had been given than in the case of the observations of Fig 3, B, and the significance of this will be discussed immediately If we were to take composites of all the curves for the fed and the starved animals respectively, it is clear that they would show that mobilization of sugar from the glycogen stores is an important factor in the recovery process Since all the animals of each group were in approximately the same condition with regard to glycogen content it is evident that no accurate method of assay can be based on a determination of the blood sugar at any period of the curves, thus confirming our previous conclusion Convulsions occurred in three of the starved animals, in one of them in 50 minutes, in another in 90 minutes, and in the third in 225 minutes One of the animals died in 1 hour after the injection, and another in 2 hours and 10 minutes The time of incidence of convulsions is therefore no accurate measure of the dose of insulin

### DISCUSSION

The features of the results of these experiments that stand out most prominently are the immediate onset in the decline in blood sugar, its practical constancy during the half hour following the injection of insulin, and the variability both in the time of onset and in the rate of recovery which afterwards occurs The rate of the initial fall is independent of the nutritional condition of the animal and, within very wide limits, the dose of insulin has no constant influence on it The prompt nature of this fall might seem to indicate that the hypoglycaemia must depend on something occurring in the blood itself, either an acceleration of blood glycolysis or a polymerization of sugar to glycogen With regard to blood glycolysis, one of us working with G S Eadie has been unable to find that insulin has any influence on the rate of glycolysis in sterile incubated defibrinated or oxalate blood, or in mixtures of blood and expressed muscle juice Neither have we found that the heating of blood or of protein free filtrates of it taken from rabbits when the hypogly

<sup>1</sup> Some of the experiments used in this series of curves were also used in the curves of Fig 1



cæmia is most pronounced causes any greater increase in reducing power than occurs under the same conditions in the normal blood of the same animal. The sugar must therefore disappear from the blood because it passes into the tissues. Insulin no doubt also passes out of the blood into the tissues, and we may suppose that when it enters the tissue cells it sets up in them some process that leads to the immediate disappearance of sugar as such, so that a "vacuum" for sugar is created in the cells and sugar is withdrawn from the blood more rapidly than the supply can be replenished. It is possible that the first step in this process is the formation of some compound between sugar and insulin and that this may occur in part in the blood itself, but it is unlikely that such an intravascular process is any essential step in the disappearance in blood sugar.

The causes for the setting up by insulin of a sugar vacuum in the cells may be either that it stimulates combustion of the sugar or that it condenses it into glycogen or reduces it to fatty acid. In the former case we should expect to find that the respiratory quotient is increased following the administration of insulin. This has definitely been shown to be the case in diabetic laboratory animals and in diabetic patients, although the rise is apparently somewhat delayed when compared with the fall in blood sugar. Dixon and Pember working in this laboratory have shown that R.Q. is also usually markedly increased in normal dogs and less definitely so in rabbits shortly after insulin is given, which would seem to show that increased combustion of sugar is an important factor in causing the decline in sugar concentration in the tissue cells and blood. It is also interesting to recall here that ketonuria is one of the first of the symptoms of diabetes to disappear under the influence of insulin<sup>(1)</sup>. Evidently the increased combustion of sugar is immediately followed by the physiological oxidation of the accumulated fatty acid metabolites (B-oxybutyric acid, etc.).

With regard to the possibility that the sugar disappears because it becomes polymerised to glycogen, it is of significance that glycogen becomes abundantly deposited in the liver of depancreated dogs when they are given sugar and insulin (although none forms with sugar alone), but whether a similar process is responsible for reduction of sugar in normal animals is not revealed by the present experiments, although it is of interest that the initial percentage of glycogen in the liver bears no relation to the rate of fall in blood sugar during the first half-hour following insulin. Whether insulin similarly stimulates glycogen formation in the muscle cells is also unknown. Hepburn and Latchford<sup>(2)</sup>

could obtain no evidence of glycogen formation in their experiments on the perfused surviving heart under the influence of insulin and it will require most careful comparison of the glycogen-content of the muscles of normal and insulin-treated animals before the question as to whether insulin stimulates glycogen formation in normal muscles can be answered. The difficulty in such an investigation depends on the fact that large total amounts of glycogen might become formed without causing a change in the percentage amount that is measurable within the experimental limits of available analytical methods.

Much attention has recently been paid by Hewitt and de Souza and Hewitt and Pryde<sup>(7)</sup> to the possibility that glucose is transformed in the tissue cells into the highly-reactive ethylene variety ( $\lambda$  glucose) as a preliminary step to its utilization in the tissues and at the suggestion of Prof. Andrew Hunter, and with his collaboration, we are at present investigating the possibility that insulin may stimulate such a change<sup>1</sup>. If glycogen formation should be stimulated by insulin in the muscles of normal animals, as undoubtedly it is in the liver of diabetic animals, it is difficult to understand why increased combustion should also occur unless we imagine that the formation of muscle glycogen is a necessary preliminary step in the transformation of glucose into the combustible ( $\gamma$ ) variety.

Turning now to the mechanism of the process by which the blood recovers its normal percentage of sugar, it is perfectly clear from the results recorded in this investigation that an important factor is the mobilization of sugar from the glycogen stores of the liver. This is most clearly demonstrated in the curves of Fig. 6. It will be observed, however, that even where there is abundance of this reserve of glycogen the rate in recovery in blood sugar may vary considerably among different animals. Other factors must be involved. One of them may be the effective dose of insulin for although this was the same per kilogram of body weight in the different animals, it may not have been the same per gram of active tissue (muscle). There is no evident way of testing this possibility for it will be seen that there is no parallelism between the actual body weight of the animals and the rate of recovery of blood sugar (cf. Fig. 6). Another factor depends on the sensitiveness of the mechanism which regulates the rate of glycogenolysis in the liver in response to the call of the blood (and tissues) for sugar. It will be observed from the glycogen percentages of the liver which are appended

<sup>1</sup> Since this paper went to press this effect of insulin has been demonstrated by Winter and Smith (*Brit. Med. Journ.*, Jan. 6, 1922)

fallen considerably below the level of .045 at which they most frequently occur with weaker doses.

9. The occurrence of convulsions cannot therefore be depended upon as an exclusive method of physiological assay.

The expenses of this research were in part defrayed by a grant from the Carnegie Corporation.

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# THE COLLOIDAL ALKALI RESERVE OF THE BLOOD.

## By T. H. MILROY.

(From the *Physiological Laboratory, Queen's University of Belfast.*)

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WITHIN recent years much important work has been done on the transport of carbonic acid in the blood, and the extensive literature dealing with this subject has been collected and summarised by van Slyke(1) and Warburg(2) amongst others. As this communication is concerned essentially with a treatment of the same subject, mainly however from the standpoint of the distribution of the base between the disperse phase and the disperse medium, it is not necessary to give full references. It is evident from the work of these two writers and from the investigations of L. J. Henderson(3), Parsons(4) and, in a slightly different form, from those of Campbell and Poulton(5) and Joffe and Poulton(6), that the transport of carbonic acid in the blood depends mainly upon the base secured from the colloidal constituents of the red cells and to a minor extent upon that obtained from the plasma proteins. In a homogeneous solution the distribution of base between two competing acids of the weak type can be readily calculated, if the base concentration, the concentrations of the competing acids, and the dissociation constants of the acids be known. Parsons especially among recent writers has dealt with this aspect of the subject from the mathematical standpoint. As the conditions in the plasma as well as in the

necessary, however, in this paper to give a few examples of these curves, as the direct titration values furnish all the information required. These conductivity measurements were made in the first place in order to have graphic records of the process of neutralisation and, in the second place, to detect any variations in the total electrolyte concentration. The curves also show the effect of ampholytes delaying the rise in  $(H^+)$  just beyond the reaction of the indicator change point, namely on the acid side of the beginning of the colour change zone. On removal of these ampholytes by dialysis, the curves no longer show this peculiarity as is evident on comparison of the graphs in Figs. 1 and 2.

Fig. 1 shows the curves for the ultrafiltrate obtained from blood from

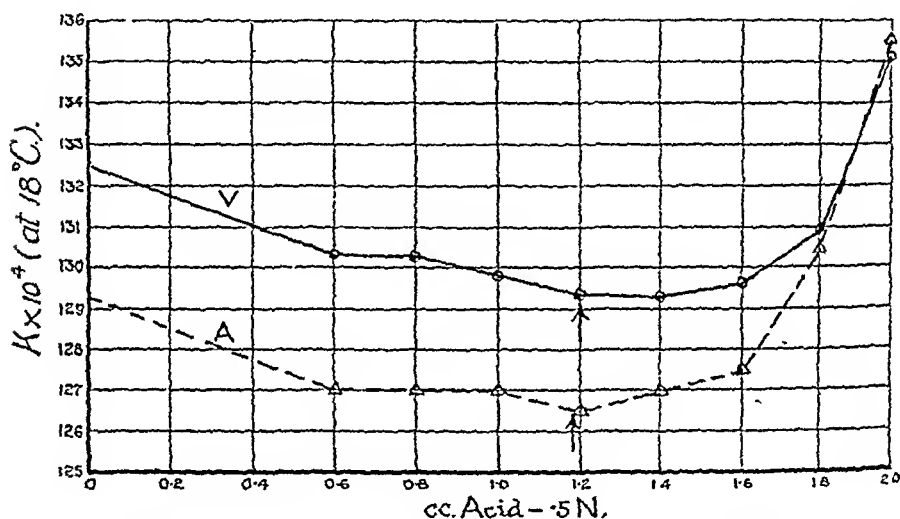


Fig. 1. 20 c.c. ultrafiltrate. A = arterial plasma. V = venous plasma.

carotid artery and jugular vein respectively. These specimens were taken and treated throughout with great care to avoid as far as possible loss of carbonic acid. The arrows give the indicator end points. The addition of .2 c.c. of .5N acid to 20 c.c. filtrate = .005N base in the disperse medium. The base of the disperse medium includes dibasic phosphate as well as the bicarbonate. The graphs of arterial and venous blood are of the same type but there are certain differences to be observed. First, the total electrolyte concentration of ultrafiltrate of the venous blood is higher than that of the arterial blood, and, second, the titration end point with the venous ultrafiltrate is rather later than with the arterial. The slightly greater alkalinity of the venous ultrafiltrate is also shown in the conductivity graphs. The base concentration of the

venous ultrafiltrate was approximately  $\cdot 002$  mol. higher than that of the arterial.

The following titration values of the alkalinity stated in terms of normality were obtained in the case of ultrafiltrates from seven different samples of blood plasma. In the first column the alkalinity of the disperse medium is given and in the second the alkalinity of the plasma corrected for the protein content in each case.

TABLE I.	Disperse medium	Plasma		Disperse medium	Plasma
(1)	$\cdot 0236$	$\cdot 0218$	(5)	$\cdot 0240$	$\cdot 0222$
(2)	$\cdot 0288$	$\cdot 0264$	(6)	$\cdot 0264$	$\cdot 0242$
(3)	$\cdot 0292$	$\cdot 0267$	(7)	$\cdot 0280$	$\cdot 0256$
(4)	$\cdot 0236$	$\cdot 0220$			

The average value for the alkalinity of the disperse medium is  $\cdot 0262$  normal, and for the original blood plasma  $\cdot 0241$  normal. Rona and György(9) have also determined the alkalinity of ultrafiltrates of blood serum and have found, after correction for the protein content, an average value of  $\cdot 0224$  normal. The variations in the alkalinity of such ultrafiltrates are in part due to differences in carbonic acid pressure in the original plasma, affecting the distribution of the base between the disperse phase and medium.

## II. *The alkali content of the disperse phase of the plasma.*

The amount of base held by the colloids of the plasma is not definitely known, although it has been recognised from the experiments of Zuntz, Rona and György and others that base can be removed from this store by the action of carbonic acid. If the fixation of base is a function of the colloidal concentration, it is evident that the amount held by the plasma in this form must be small compared with the quantity bound to the colloids in whole blood, as the latter must be nearly three times as great as the former. Apart from this, hæmoglobin and still more oxyhæmoglobin might in virtue of a possibly higher acid dissociation constant secure more base than the plasma colloidal ampholytes. The method which was mainly employed to determine the plasma colloidal base was in general terms the following:

Two samples of the same plasma were taken. One was diluted with a definite volume of  $\cdot 85$  p.c. NaCl, while to the other there was added the same volume of a boric acid-mannitol solution of sufficient strength to combine with all the base united to the colloids and weak acids of the plasma. The alkalinity of the two ultrafiltrates was then determined, the difference giving the amount of base originally united with colloid.

The addition of mannitol to a boric acid solution raises the ionisation constant of the acid to  $1 \cdot 10^{-5}$  (Magnanini(10)). Such an acid will displace the base from all the weaker plasma acids. The borate so formed passes into the ultrafiltrate, and, on titration with a strong acid ( $\cdot 5N$ .  $H_2SO_4$ ) to the methyl orange end point, the full alkali value of the salt can be determined. That is to say the mannitol borate behaves in the same way as the simple borate when titrated against a strong acid. The alkalinity of the ultrafiltrate of the plasma diluted with saline is due to the bicarbonate and dibasic phosphate, while that of the boric acid ultrafiltrate is due to the base derived from these sources and from the plasma colloids. Excess of boric acid does not interfere with the reaction. The following example gives the details in a particular case.

(1) To 100 c.c. of plasma from oxalated blood 50 c.c.  $\cdot 85$  p.c. NaCl were added.

(2) To 100 c.c. of the same plasma 10 grms. mannitol were added then 26 c.c. of  $\cdot 59$  molar boric acid<sup>1</sup> in  $\cdot 85$  p.c. NaCl and the mixture made up to 150 c.c. with  $\cdot 85$  p.c. NaCl. The solution was then  $\cdot 1$  molar as regards boric acid and in the presenece of the mannitol was more than sufficient to neutralise the free bicarbonate and dibasic phosphate and remove all the base from the colloids. The ultrafiltrates of these two samples were then obtained and titrated at the same time against  $\cdot 5N$   $H_2SO_4$  to the same methyl orange end point.

The results of four such experiments are given in Table II, correction being made for the dilution.

TABLE II.

	Before	After boric acid-mannitol	Base increment
(1)	$\cdot 0258$	$\cdot 0368$	$\cdot 0110$
(2)	$\cdot 0300$	$\cdot 0380$	$\cdot 0080$
(3)	$\cdot 0298$	$\cdot 0383$	$\cdot 0085$
(4)	$\cdot 0247$	$\cdot 0360$	$\cdot 0113$

The base increment therefore in the disperse medium produced by the removal of the colloidal base of the plasma was on an average slightly below  $\cdot 01$  normal. Variations in the increment are in part due to the initial distribution of the base between the colloids and the disperse medium which is determined by the concentration of the other competing acids, carbonic and to a slight extent phosphoric acid.

Other methods may be employed for the removal of base from the plasma colloids. For example the alkalinity of the ultrafiltrates obtained from plasma before and after saturation with carbonic acid may be determined. This was done in the case of four series of ultrafiltrates obtained from different samples of blood.

<sup>1</sup> All boric acid solutions were made from Kahlbaum's boric anhydride (for analysis).

TABLE III.

	Before CO <sub>2</sub>	After CO <sub>2</sub>	Base increment
(1)	·024	·034	·010
(2)	·030	·039	·009
(3)	·029	·037	·008
(4)	·032	·038	·006

These values refer to disperse medium and are therefore not corrected for the colloid content of the plasma. The base increments correspond closely to those obtained in the boric acid-mannitol experiments and hence it is probable that practically all the colloidal base can be removed by carbonic acid. Rona and György<sup>(9)</sup> determined the alkalinity of ultrafiltrates of blood serum before and after CO<sub>2</sub> and obtained values for the alkali increment higher on the whole than those given above, ranging from ·0095–·018 normal.

In a series of experiments described by Hasselbalch and Warburg<sup>(11)</sup> in which serum was equilibrated with carbonic acid at gradually rising pressures, the volume of combined CO<sub>2</sub> was determined from about 40 mm. to 710 mm. pressure. These writers regard all carbonic acid fixed at reactions on the acid side of the probable isoelectric zone of the colloidal ampholytes as bound to these ampholytes acting as bases. It is however by no means certain that immediately on the acid side of this zone, the colloidal ampholytes are entirely freed from base by such an acid as carbonic acid. If we regard, as we are entitled to do, all the carbonic acid fixed at a partial pressure of 40 mm. as being in the form of bicarbonate in the disperse medium and if further we regard the increase which occurs from that point up to 710 mm. as provided by base removed from the disperse phase of the serum, then the increment in fixed carbonic acid (Table III of their paper) transformed into terms of normality corresponds to a ·0097 molar rise in bicarbonate in the CO<sub>2</sub> serum. It is evident that as the CO<sub>2</sub> pressure falls, the base secured by the free carboxyl groups of the colloidal ampholytes rises, and, if the concentration of these ampholytes is high, the bicarbonate concentration in the disperse medium may fall to a low level as in the case of whole blood.

The alkali content of the disperse phase of the plasma may also be studied in dialysed specimens (see Section IV).

### III. *The alkalinity of the disperse medium and of the disperse phase of the laked whole blood.*

In order subsequently to determine the amount of base fixed to the colloids in the whole blood, it is necessary in the first place to obtain



information regarding the alkalinity of the disperse medium of the whole blood. The whole blood was examined after hæmolysis in order to facilitate comparison between the plasma alone and the plasma plus the cellular constituents. Volume determinations of the cells and estimations of the approximate protein value of the laked blood were also made.

*Example 1.* The blood contained 38 p.c. cells, 62 p.c. plasma and the nitrogen content of the whole blood corresponded approximately to 18.3 p.c. protein.

(1) 100 c.c. of the whole blood were taken, 1 gram saponin added and the mixture made up to 150 c.c. with .85 p.c. NaCl. After thorough shaking the blood was completely hæmolysed. The laked blood was then filtered, and the alkalinity of the ultrafiltrate determined. After correction for the dilution and for the protein content this was found to be .0263 molar.

(2) To 100 c.c. of the plasma (8 p.c. protein) of the same blood one gram saponin was added in order to make the conditions similar to those in the laked blood, and the mixture made up to 150 c.c. with .85 p.c. saline.

The alkalinity of the plasma ultrafiltrate after correction for the dilution and protein content was .0297 molar. Therefore the alkalinity of 1000 c.c. laked blood was made up of .0184 mol. free base in the plasma, .62 (.0297); and .0079 mol. free base from the cells. The approximate free base concentrations in this specimen were:

				Mol. base
In 1000 c.c. laked blood	...	...	...	.0263
„ plasma of 1000 c.c. laked blood	...	...	...	.0184
„ cells of „	„	„	...	.0079
„ 1000 c.c. plasma	...	...	...	.0297
„ „ cells	...	...	...	.0207

These values represent all the titratable free base whether as bicarbonate or dibasic phosphate or attached to other weak acid groups, and are therefore higher than alkalinity values determined from estimations of combined  $\text{CO}_2$ .

*Example 2.* This sample was taken from an old horse in very poor condition and the cell content was found to be very low (17.5 p.c.). Laked blood and plasma were examined as in the preceding sample. The protein content of the whole blood was 16.99 p.c. and that of the plasma 7.437 p.c. After correction for the dilution and protein content, the laked blood ultrafiltrate was found to be .0250 m. base, the plasma .0267 m. Therefore the distribution was

				Mol. base
In 1000 c.c. laked blood	...	...	...	.0250
„ plasma of 1000 c.c. laked blood	...	...	...	.0220
„ cells	„	„	...	.0030
„ 1000 c.c. plasma	...	...	...	.0267
„ „ cells	...	...	...	.0171

The methods referred to for the determination of the colloid base of the plasma can also be made use of in the case of laked blood. The boric acid-mannitol method may be employed for the determination of the base attached to the colloids in laked blood either in the presence or absence of the alkali of the disperse medium. In this section the colloid base of the undialysed laked blood will be dealt with.

*Exp. 1* (1) 100 c.c. whole blood were taken. To this 1 gram saponin and 85 p.c. NaCl up to 150 c.c. were added.

(2) To 100 c.c. of same blood, 1 gram saponin 26 c.c. boric acid (.59 mol.), 10 gm mannitol, and .85 p.c. NaCl up to 150 c.c. were added.

The protein content of the whole blood was approximately 18 p.c. and the cell volume 40 p.c. The ultrafiltrate of (1) after correction for dilution and for the removed protein had an alkalinity value of .0263 molar base, while that of (2) showed a rise in alkalinity up to .0513 molar. The increment in (2), namely, .025 molar, was derived from the colloid base of the whole blood.

In another sample of laked blood in which a volume determination of the cells was not made but in which the protein content was 17 p.c., the values were—before boric acid-mannitol, .0245, after boric acid-mannitol, .0490, or a base increment in the disperse medium of .0245 molar.

In these two samples of blood the maximal amount of base removable from the disperse phase raised the alkalinity of the blood to .05 molar approximately. This consisted of the free base originally present plus the colloid base, the actual increment depending upon the amount originally secured by carbonic acid from the colloid base prior to the action of the stronger acid. It may be taken for granted that boric acid-mannitol in the strength used (.1 molar) displaces all the weak acid radicles, carbonic and colloidal carboxyl groups. It is interesting to compare the figures given by Hasselbalch and Warburg<sup>(11)</sup> (Table III) for defibrinated ox blood exposed to varying CO<sub>2</sub> pressures.

If these figures be calculated as molar bicarbonate values, between 43.5 mm. and 692.9 mm. CO<sub>2</sub> pressure the fixed CO<sub>2</sub> rises from .018 to .045 molar. At the lower pressure all the fixed carbonic acid is as bicarbonate, and at the higher, according to these investigators partly as bicarbonate in the disperse medium and partly fixed by the colloidal ampholytes acting as bases. Regarding all as bicarbonate even at the high pressure, the increment in fixed CO<sub>2</sub> corresponds approximately to the maximal amount of base obtainable from the colloids of the defibrinated blood.

IV. *The alkali content of the disperse phase of blood plasma and of laked blood after dialysis against .85 p.c. NaCl.*

If blood plasma be dialysed against water, the less soluble globulin portion separates out and there is a gradual loss of alkali from the disperse phase owing to hydrolysis. When blood plasma is dialysed against .85 p.c. NaCl the globulin remains in solution and the colloid base is not withdrawn even when dialysis has gone on for some days. During the dialysis of the plasma a small quantity of the base of the disperse medium becomes attached to the colloids. With the removal of other competing acids, carbonic acid, etc. the colloidal ampholyte COOH groups secure more base than under the conditions normally existing in the blood. The electrostatic attraction between the cations and the non-diffusible colloidal anions also prevents, so long as hydrolysis does not occur, a loss of the former.

Experimental evidence in support of the retention of colloid base during dialysis against .85 p.c. NaCl can readily be obtained. For example the amount of base which can be removed by boric acid mannitol from plasma dialysed against .85 p.c. NaCl is rather greater than that obtained by the action of the same acid on the colloids of undialysed plasma. That is to say part of the alkali of the disperse medium during dialysis is fixed by the colloidal ampholytes. In the second place, if plasma be dialysed against .85 p.c. NaCl and then bicarbonate added to give a .04*N* concentration, the ultrafiltrate of this solution shows no lowering in the alkali value, such as would occur had there been a removal of base from the dialysed colloids and a replacement of this loss from the added bicarbonate.

From Fig. 2 it will be seen that the sharp rise in conductivity occurs .1 c.c. before the indicator end point. An addition of .8 c.c. *N* acid to 20 c.c. ultrafiltrate corresponds to the theoretical alkali value of a .04 normal solution.

*Method of determining the colloid base in dialysed plasma.* Collodion tubes of 200 c.c. capacity were filled with blood plasma. These were fitted with rubber stoppers to which mercury manometers were attached. They were then placed in large vessels containing .85 p.c. NaCl, the external fluid being frequently changed. The saline solutions were always made up with conductivity water. Thymol was added to the inner and outer fluids. After 48 hours' dialysis the plasma was examined as follows. The protein content was determined in a small sample. Two portions, each of 100 c.c. dialysed plasma were taken, to one 50 c.c. saline were

added and to the other 50 c.c. saline containing boric acid and mannitol, the concentration of the acid in the latter being .1 molar.

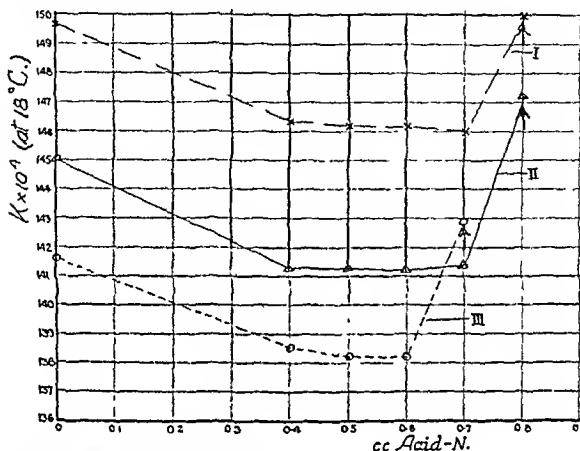


Fig 2. Neutralisation conductivity curves of

I, .04 *m*,  $\text{NaHCO}_3$  in .85 p.c.  $\text{NaCl}$ .

II, the ultrafiltrate obtained from blood plasma which had been dialysed for 24 hours against .85 p.c.  $\text{NaCl}$  and then sodium bicarbonate added up to .04 molar.

III, the ultrafiltrate obtained from blood plasma which had been dialysed for 24 hours against water and then made up to .04 mol.  $\text{NaHCO}_3$  and .85 p.c.  $\text{NaCl}$ . The arrows give the indicator end points, .8 c.c. *N* acid for 20 c.c. I and II ultrafiltrates and .7 c.c. for ultrafiltrate III.

The ultrafiltrates of these two portions were then obtained and the alkalinity in each determined. The ultrafiltrate of the dialysed plasma plus saline was free from alkali, while that of the other showed an alkalinity equal to .0092 molar. As the dialysed plasma contained 8.5 p.c. protein and the dilution was 100 to 150, this alkalinity corresponded to .0129 mol. base removed from 1000 c.c. of dialysed blood plasma undiluted, and corrected for the volume alteration due to colloid removal.

Similar estimations were made in four other samples of dialysed blood plasma, the following increments in base being obtained, .011, .013, .009 and .012 mol. The protein percentage of these four specimens were respectively 7.8, 8.4, 6.8 and 8.6. The amount of base therefore

obtainable from the colloids present in 1000 c.c. of dialysed plasma was approximately  $\cdot 012$  mol. This value is slightly higher than the base increments obtained from the disperse phase in the undialysed plasma (Sect. II).

*Laked whole blood.* Collodion tubes were filled with 200 c.c. of oxalated whole blood and dialysed, in the first place without laking, against large quantities of  $\cdot 85$  p.c. NaCl, frequently changed. The tubes were often shaken. After 24 hours saponin was added to the contents of the tubes and after thorough admixture, the tubes with the laked blood were replaced in fresh  $\cdot 85$  p.c. NaCl. Dialysis was continued for another 24 hours with frequent changes of the external fluid. The ultrafiltrates obtained from dialysed laked blood, unlike those from dialysed plasma, always contained traces of alkali, so that correction required to be made for this in the determinations of the colloid base.

Two samples of dialysed laked blood were always compared, one to which boric acid-mannitol was added and the other without this addition. As in the case of dialysed plasma 100 c.c. portions were taken, one diluted with 50 c.c. saline and the other with 50 c.c. boric acid-mannitol saline. The following two examples may be given.

(1) The blood contained 30.2 p.c. cells. The protein content of the laked blood after dialysis was 18.65 p.c. The ultrafiltrate of the diluted dialysed blood to which no boric acid was added had an alkalinity equal to  $\cdot 002$  m., while the diluted sample to which boric acid-mannitol had been added furnished an ultrafiltrate of  $\cdot 027$  mol. base.

This corresponds to  $\cdot 0328$  mol. base attached to the colloids in 1000 c.c. of the undiluted laked blood, correction being made for the colloids removed in ultrafiltration. That is to say  $\cdot 0328$  mol. base was removed from 186.5 gm. protein.

(2) In a similar experiment the protein content of the dialysed laked blood was 18.37 p.c. On comparing the ultrafiltrate from the dialysed laked blood which had been acted upon by boric acid-mannitol, with the ultrafiltrate from a similar specimen to which no acid had been added, the former showed after correction for dilution, etc., an increase in alkalinity corresponding to  $\cdot 0269$  molar base.

Thus after the removal of the free base by dialysis, boric acid-mannitol can remove from the colloids present in 1000 c.c. of laked blood approximately  $\cdot 027$ – $\cdot 033$  mol. base. Approximately thrice as much base may be obtained from the colloids of the whole blood as can be secured from the plasma colloids. The following example is more or less typical.

In a blood containing 30 p.c. cells, the colloid base of 1000 c.c. whole blood amounted to  $\cdot 03$  mol., and the colloid base of 1000 c.c. plasma of same blood was  $\cdot 012$  mol. Then if the  $\cdot 03$  mol. base in the dialysed whole blood comes  $\cdot 0084$  mol. from plasma and  $\cdot 0216$  from the cells, the preponderant colloid base content of the cells is evident.

In this case it must have amounted to  $\cdot 072$  mol. per 1000 c.c. cells, or a value six times greater than the colloid base value of the plasma. Practically the sole source of the colloid base of the cellular elements is the hæmoglobin present in the red corpuscles.

*V. The distribution of the base of the disperse phase of the plasma and of laked blood between the colloids and foreign competing acids.*

In the preceding sections dealing with the colloids of the plasma and whole blood, attention was solely directed to the maximal amount of base removable by competing acids. In this section the subject of the distribution of the base between the colloidal ampholytes of fixed concentration and competing acids of varying concentration will be dealt with.

The study of the base distribution in normal blood between the cell colloids and carbonic acid is beset with many difficulties. In the first place the red cells are regarded usually as being impermeable for cations while permitting free exchange of anions, so that a rise in the ( $\text{HCO}_3$ ) concentration of the plasma is associated with a fall in the ( $\text{Cl}$ ). In the second place there are very ill-defined and variable volume alterations in the cells produced by carbonic acid and it is almost impossible to determine accurately the influence exerted by this factor in the base distribution between the disperse medium of the cells and plasma. In the third place the variations in the  $\text{NaCl}$  content in the cells and plasma and their effect on the activity coefficients of such weak competing acids as the colloidal ampholytes and carbonic acid have not yet been studied with sufficient care. For these reasons amongst others attention has been directed to the much simpler conditions existing in laked blood and in plasma which have been dialysed against  $\cdot 85$  p.c.  $\text{NaCl}$ . Also instead of using carbonic acid as the competing acid, one with a slightly higher dissociation constant was chosen, namely cacodylic acid. With such an acid, solutions of a definite molar concentration could easily be prepared and the distribution of the total colloid base (previously determined by boric acid-mannitol) between the two acids could readily be studied. Some experiments were also carried out with boric acid, a much weaker acid than cacodylic, in order to see whether the base distribution between cacodylic acid and the colloids, and boric acid and the colloids followed the same laws. The acid dissociation constant of cacodylic acid is given by Lunden<sup>(12)</sup> as  $6\cdot 4 \cdot 10^{-7}$  at  $25^\circ$  and that of boric acid as  $6 \cdot 10^{-10}$  at  $18^\circ$ . The question of the distribution of base between these acids and

the colloids of plasma and blood will be considered theoretically after the experimental results have been given.

*The action of cacodylic acid on dialysed blood plasma.* Five collodion tubes, each containing 200 c.c. oxalated horse blood plasma, were dialysed against .85 p.c. NaCl for 48 hours. The contents of the tubes were then mixed and the nitrogen (protein) determined in two portions (2 c.c.), the nitrogen value was 1.435 p.c. (8.968 p.c. protein). A solution of cacodylic acid 4.14 gm. in 100 c.c. .85 p.c. NaCl was prepared. 5 c.c. of this solution made up to 150 c.c. gave a .01 molar concentration. The dialysed blood plasma was treated with cacodylic acid as follows:

1. 100 c.c. plasma + 2.5 c.c. of the cacodylic acid solution (c.a.) + .85 p.c. NaCl up to 150 c.c. = .005 m. (c.a.).
2. 100 c.c. plasma + 5 c.c. c.a. up to 150 c.c. = .01 m.
3. " " + 7.5 " " = .015 m.
4. " " + 12.5 " " = .025 m.
5. " " + 20 " " = .04 m.
6. 100 c.c. plasma + 50 c.c. saline.
7. " " + 50 c.c. boric acid-mannitol saline.

The ultrafiltrates of these solutions were then obtained and alkalinities determined.

In the first place the ultrafiltrate of the diluted dialysed plasma (*i.e.* 6) was found to be free from base, and in the second place the amount of base removed by boric acid-mannitol (*i.e.* 7) was .011 mol. That is to say the maximal amount of base removable from the colloids of dialysed plasma by .1 molar boric acid-mannitol was .011 mol. Table IV (below) gives the amount removed by cacodylic acid in the different concentrations. Other experiments gave practically the same results.

*The action of boric acid on dialysed blood plasma.* Two series were carried out with boric acid acting on two different samples of dialysed plasma. As this acid is so much weaker than cacodylic, it required to be used in higher concentrations. The results owing to the small amount of base removed are less satisfactory than those obtained with cacodylic acid. This especially holds for low concentrations of the acid. The protein content of (1) was 8.1 p.c. and of (2) 6.9 p.c.

Boric acid therefore acting in .3 molar concentration removes only 32 p.c. (1) and 33.3 p.c. (2) of the total available colloïd base of the

TABLE IV.

Cacodylic acid (mol.)	Base removed (mol.)
0	0
.005	.0045
.010	.0068
.015	.0076
.025	.0095
.040	.0104
(Boric acid-mannitol .1 mol.)	.0110

TABLE V.

Boric acid (mol.)		Base removed (mol.)	
(1)	(2)	(1)	(2)
0	—	0	—
.01	—	.0015	—
.03	—	.0022	—
.05	—	.0025	—
.10	.10	.0030	.0022
.30	.30	.0048	.0040
(Boric acid-mannitol .1 mol.)		.0150	.0120

plasma, while cacodylic acid even in a concentration of .025 molar removes over 86 p.c. of the available base.

Experiments similar to those just described for plasma were now carried out with dialysed laked whole blood. The usual method of dialysis against .85 p.c. NaCl was employed. One sample of this was filtered without the addition of any acid in order to find out whether the dispersc medium was free from base. Ultrafiltrates obtained from laked blood dialysed against .85 p.c. NaCl for 48 hours with frequent changes always contained traces of alkali. The alkali value of the ultrafiltrate obtained in this way requires to be deducted from the alkali values of the ultrafiltrates obtained from blood to which the competing acid had been added. The values given in the table denote the actual base increments produced by the action of the competing acid on the colloids of laked blood. The blood referred to in Table VI contained slightly over 30 p.o. cells and the protein content of the dialysed laked blood was 18.37 p.c.

Tables VI and VII give the base removed from dialysed laked blood by cacodylic acid and boric acid respectively.

Another sample of blood was used for the boric acid series.

TABLE VI.

	Cacodylic acid (mol.)	Base removed (mol.)
(1)	.01	.0080
(2)	.02	.0130
(3)	.03	.0200
(4)	.04	.0240
(5)	.05	.0247
(6)	.06	.0255
(Boric acid-mannitol .1 mol.)		.0270

TABLE VII.

	Boric acid (mol.)	Base removed (mol.)
(1)	.02	.0023
(2)	.04	.0032
(3)	.06	.0048
(4)	.10	.0060
(5)	.30	.0082
(Boric acid-mannitol .1 mol.)		.0201

Of the total available colloidal base cacodylic acid in .04 mol. concentration secures about 89 p.c. while boric acid in the same concentration slightly over 10 p.c. It is important to note that with a .02 molar concentration of cacodylic acid, the base is nearly equally shared between the colloids of the dialysed laked blood and the foreign acid.

#### VI. *Comparison between the observed and the theoretical values of the base distribution between competing acids.*

In van Slyke's recent paper (13) on buffer values there is an interesting discussion of the relationship existing between increment in base and increment in pH under conditions similar to those existing in blood. At a defined pH the ratio of the free acid to the anion concentration is



determined by the value of the dissociation constant of the acid concerned. Taking the average acid dissociation constant for the colloidal ampholytes as being equal to the  $(H^+)$  of normal blood, the ratio of the anion to the undissociated acid is unity. Information regarding the amount of base set free in the blood within a narrow range of reaction has been almost entirely obtained from studies which are in the main theoretical and are based on the Henderson equation or some mathematical modification of the same.

van Slyke has made use of the differential ratio  $\frac{dB}{dpH}$  to study the amount of base fixed in the disperse medium and the disperse phase. For buffers other than bicarbonate, the most important being the protein buffers, the differential ratio  $\frac{dB}{dpH} = \frac{-dBHCO_3}{dpH}$ , as the increase in the base secured by the colloidal ampholytes is equal to the fall in the  $HCO_3$  value. The amount of base secured by the colloidal acids for a given concentration will naturally vary with the value taken for the average dissociation constant. From a consideration, mainly theoretical, of this subject, van Slyke concludes that at the blood reaction about .018 n. base is attached to the colloidal ampholytes and the total alkali bound by all the weak buffer acids as approximately .04 n.

In this communication an endeavour has been made to arrive at a knowledge of the base distribution of the blood through a different channel of approach.

Even although the laws which govern the base distribution between two competing acids in homogeneous solutions may not hold good in their entirety for heterogeneous ones still it is better in order to gain a first approximation to the truth to regard the latter from the same standpoint as the former. Sørensen has emphasised the importance of regarding colloidal (emulsoid) solutions from the purely chemical or physico-chemical standpoint as well as from the more limited colloid-chemical one. Too little stress is laid on the chemistry of the disperse phase in its relation to that of the disperse medium.

Hence the following theoretical treatment follows the same lines that have been adopted in the consideration of the "avidities" of acids for base in homogeneous solutions (Abegg(14)). The competing acids are weak and therefore may be regarded as obeying Ostwald's dilution formula and the salts of the acids with the base are taken as completely dissociated while the acids themselves remain practically undissociated.

In a litre of the solution there are mol. base,  $c_1$  mol. of acid 1 ( $HA_1$ ), and  $c_2$  mol. of acid 2 ( $HA_2$ ). The concentration of acid 1 is fixed, that of

acid 2 is varying. Under the conditions existing  $b < C_1 + C_2$ , that is to say there is insufficient base to neutralise both acids.

Now let  $X$  = fraction of each mol. base reacting with acid 1, and  $1 - X$  then equals fraction falling to acid 2.

The salt of acid 1 ( $BA_1$ ), and of acid 2 ( $BA_2$ ) may be stated in terms of  $x$ , as  $bx$  and  $b(1 - x)$  respectively, and these also give the anion concentrations if dissociation of the salts is complete. The free undissociated acid remainders are, in the case of acid 1,  $C_1 - bx$  and of acid 2,  $C_2 - b(1 - x)$ . Then the mass law equations for acids 1 and 2 may be written

$$K_1 = \frac{[H^+][A_1^-]}{[HA_1]} = \frac{[H^+]bx}{C_1 - bx} \dots\dots\dots(1),$$

$$K_2 = \frac{[H^+][A_2^-]}{[HA_2]} = \frac{[H^+]b(1 - x)}{C_2 - b(1 - x)} \dots\dots\dots(2),$$

and 
$$\frac{K_1}{K_2} = K = \frac{x}{1 - x} \times \frac{C_2 - b(1 - x)}{C_1 - bx} \dots\dots\dots(3),$$

or 
$$x_2 + x \left( \frac{C_2}{b(1 - K)} + \frac{KC_1}{b(1 - K)} - 1 \right) - \frac{KC_1}{b(1 - K)} = 0 \dots\dots\dots(4).$$

On solving this quadratic equation, the distribution ratio of base,  $\frac{x}{1 - x}$ , between the two acids is obtained.

This equation has been made use of to study the distribution of base between the colloidal ampholytes of plasma and blood and a competing acid under the simplest and most easily reproduced conditions. The plasma and laked blood were dialysed free of all salts but sodium chloride and that was present in a definite concentration. .85 p.c. Making use of the terms previously mentioned.

$C_1$  = the concentration of the carboxyl groups in the colloidal ampholytes with a dissociation constant  $K_1$ .

$C_2$  = the concentration of the competing acid with dissociation constant  $K_2$ .

$b$  = molar concentration of available base.

$K$  and  $x$  have the significance previously mentioned.

Although there are possibly free COOH groups present in the colloidal ampholytes in dialysed plasma and blood, in the calculations which follow,  $C_1$  is taken as being equal to  $b$ . During the process of dialysis against NaCl with the removal of carbonic acid, the alkalinity of the disperse medium rises so that neutralisation of any free carboxyl groups in the colloids will probably occur.

The average acid ionisation constant taken for the colloidal ampholytes is based on van Slyke's calculations for oxyhæmoglobin and

reduced hæmoglobin. Simply for purposes of comparison the  $K$  (acid) of the plasma ampholytes and of the laked blood ampholytes ( $K_1$ ) is taken as being the same ( $6.6 \cdot 10^{-8}$ ) giving with  $K$  (acid) of cacodylic acid ( $K_2$ )  $K = 1$ .

(1) Comparison between the theoretical and observed values of the base distribution between (a) the colloids of dialysed plasma ( $C_1$ ), (b) the colloids of dialysed laked blood ( $C_1$ ) and cacodylic acid ( $C_2$ ).

Where in (a)  $C_1 = 0.11$ ;  $b = 0.11$ ; and  $C_2$  varies.

In this case for ease in calculation  $k_1$  is taken as  $6.4 \cdot 10^{-8}$  and

$$K = \frac{K_1}{K_2} = \frac{6.4 \cdot 10^{-8}}{6.4 \cdot 10^{-8}} = 1:$$

and in (b)  $C_1 = 0.27$ ;  $b = 0.27$   $C_2$  and  $K$  as above.

(a) *Dialysed blood plasma and cacodylic acid.* Table VIII gives the theoretical and observed values.

TABLE VIII.				
$C_2$	$x$	$1 - x$	$b(1 - x)$ (theoretical)	$b(1 - x)$ mol. cacodylate (actually found)
0.005	0.596	0.414	0.046	0.045
0.010	0.298	0.722	0.079	0.069
0.015	0.144	0.856	0.094	0.086
0.020	0.091	0.909	0.099	0.097
0.030	0.050	0.949	0.104	—
0.040	0.035	0.965	0.106	0.104

$b = 0.11$  (actually found).

(b) The same calculation can be carried out for cacodylic acid and dialysed laked blood where  $C_1$  and  $b$  are taken as 0.27, the other letters having the same significance as in the preceding case.

$$\frac{K_1}{K_2} \text{ or } K = 1.$$

TABLE IX.				
$C_2$	$x$	$1 - x$	$b(1 - x)$ (theoretical)	$b(1 - x)$ (found)
0.01	0.648	0.352	0.095	0.080
0.02	0.368	0.632	0.170	0.130
0.03	0.203	0.797	0.215	0.200
0.04	0.126	0.874	0.236	0.240
0.05	0.089	0.911	0.246	0.247
0.06	0.068	0.932	0.251	0.255

$b = 0.27$  (actually found).

The values of  $b(1 - x)$  found for the lower concentrations of cacodylic acid are somewhat variable, but, at the higher, similar experiments gave practically identical results.

(2) The same theoretical treatment can be extended to the boric acid competition with the blood colloids for base, but in this case the

competing acid is much weaker than the colloidal ampholytes, and therefore any possible free COOH groups in the colloids will make their influence felt at the low boric acid concentrations. For this reason among others, the boric acid observed results cannot be so readily compared with the theoretical

In order however that the two acids (caecodylic and boric) may be compared as regards their power of withdrawing base from the colloidal ampholytes it is necessary to regard them as acting under similar conditions. As regards boric acid competition for the colloidal base of dialysed plasma (a) the conditions in the specimen examined were as follows

$b = 0.12$ ,  $C_1 = 0.12$ ,  $C_2 = \text{varying boric acid conc}$   $K_1$  taken as  $6 \times 10^{-8}$  for ease in calculation

$$\frac{K_2}{K_1} = \frac{6 \times 10^{-8}}{6 \times 10^{-10}} = 100 = K$$

TABLE X

$C_2$	$x$	$1-x$	$b(1-x)$	
			m borato (theoretical)	Observed
01	017	083	00009	0015
02	883	117	0014	—
03	858	142	0017	0022
04	837	163	0019	—
05	810	181	0021	0025
10	752	248	0020	0030
30	619	381	0046	0048

( $b = 0.12$  observed)

(b) Boric acid and laked blood under following conditions

$b = 0.3$ ,  $C_1 = 0.3$   $C_2$  varying,  $K = 100$

TABLE XI

$C_2$	$x$	$1-x$	$b(1-x)$	
			Theoretical	Observed
01	047	053	00159	—
02	926	074	00222	0023
03	909	091	00273	—
04	895	105	00315	0032
05	883	117	00351	—
10	837	163	00489	0061
30	733	267	00801	0082

( $b = 0.30$  observed)

It is evident from a comparison of the theoretical and observed values of  $b(1-x)$  both for caecodylic acid and boric acid under the conditions occurring in dialysed plasma and laked blood that the distribution of the available base in the main conforms to the same laws which are known to hold for homogeneous solutions

## SUMMARY.

1. A method for the study of the disperse medium and disperse phase of the plasma and laked blood is described.
2. The alkalinity of the disperse medium of plasma is approximately  $\cdot 024$  m.
3. The alkali content of the disperse phase of the plasma is approximately  $\cdot 01$  m.
4. The alkalinity of the disperse medium of laked whole blood is less than that of the plasma of the same blood.
5. The alkali content of the disperse phase of laked blood (undialysed) is approximately  $\cdot 025$  m., but after dialysis against  $\cdot 85$  p.c. NaCl rather more is fixed by the colloids.
6. The distribution of the colloid base of the plasma and laked blood between the acid colloid groups and two foreign competing acids, cacodylic and boric acids, has been studied experimentally and theoretically.

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# THE CARBON DIOXIDE PARTIAL PRESSURE IN BODY CAVITIES AND TISSUE SPACES UNDER VARIOUS CONDITIONS. BY J. ARGYLL CAMPBELL.

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THE experiments described herein were carried out mainly to determine the effects on the  $\text{CO}_2$  partial pressure in some of the tissues and body cavities, produced by changes of temperature, muscular exercise and artificial respiration.

*Previous researches.* The previous work on  $\text{CO}_2$  partial pressure in tissues and body cavities is very extensive. Amongst the earliest researches are those of Davy(1) in 1823, on the  $\text{CO}_2$  content in the gases in a case of pneumothorax and those of Leconte and Demarquay(2) in 1859 on the changes in  $\text{CO}_2$  content in gases injected subcutaneously and into the peritoneal cavity. Attention will be directed here to some of the researches of physiological significance and of importance to the present work. In 1914 Webb, Gilbert, James and Havens(3) injected nitrogen into one side of the thoracic cavity of one monkey and a similar amount of air into one side of the thoracic cavity of another monkey of about the same size and weight, and found the  $\text{CO}_2$  percentages to be 8.52 and 8.06 respectively after 48 hours. Rist and Strohl(4) concluded that there is a balance between the gases of a pneumothorax cavity and the gases in the venous blood; whilst Dautrebande and Spehl(5) pointed out that it was more correct to state that the composition of the gases was regulated by the blood bathing the compressed lung, this being richer in  $\text{CO}_2$  than that of the normal lung, because of the lessened circulation due to the compression. Grass and Meiners(6) distinguished between an open and closed pneumothorax by drawing out some of the gas from the cavity. If there was an opening elsewhere communicating with the air, fresh air rushed in and thus lowered the  $\text{CO}_2$  percentage. They also described a method to estimate the volume of gases in the thoracic cavity.

Henderson and Haggard(7) injected air into the abdominal cavity of dogs under local anaesthesia and showed that the  $\text{CO}_2$  tension in the abdominal air approximated in about one hour, to that in the arterial

body in general, such as an increase of metabolism accompanied by a diminished excretion of  $\text{CO}_2$ . General heating may have dilated the capillaries and brought far more blood to the skin so that the circulation time of the blood was prolonged. Food did not produce any appreciable change in the  $\text{CO}_2$  partial pressure under the skin of a cat.

*Effect of exercise.* Table VI records results showing that exercise for 18 minutes markedly increased the  $\text{CO}_2$  partial pressure under the skin in the case of a rabbit although the body temperature was not altered greatly. Other experiments with rabbits showed similar results. In Table VII results are given which were obtained from a kitten. Exposure to a warm atmosphere and two periods of ten minutes mild exercise increased the  $\text{CO}_2$  tension under the skin from 40 mm. to 48 mm.; subsequent rest in a cooler room reduced the tension to 40 mm. The author hopes to carry out similar experiments on man during exercise and exposure to heat.

*Effect of artificial respiration.* Artificial respiration was performed under urethane by means of Schuster's<sup>(12)</sup> double-action pump, made for Dale and Evans<sup>(13)</sup>. The  $\text{CO}_2$  partial pressure under the skin before artificial respiration was commenced, in the experiment illustrated in Table VIII, was about 50 mm.; such high figures were sometimes observed with the animal under an anæsthetic. After 21 minutes of artificial respiration the tension fell to 37 mm. but was not altered much by a further 60 minutes of artificial respiration. Except for a few short interruptions, the artificial respiration was continuous. About 22 c.c. of air were pumped into and withdrawn from the lungs 190 times a minute. After cessation of artificial respiration the  $\text{CO}_2$  tension rose again and reached 54 mm. In this experiment the respiratory quotient was 0.6 during artificial respiration, so that the lung was probably not completely opened up by the pumping in and out of 22 c.c. of air. In an experiment on a cat (see Table IX) the amount of air pumped into the lungs was much greater and the  $\text{CO}_2$  tension under the skin fell as low as 21 mm. In this experiment the respiratory quotient was above 1. Many other experiments, in some of which air was injected into the abdominal cavity, gave similar results<sup>1</sup>. The blood pressure was recorded in some of these

<sup>1</sup> Further experiments, carried out by the author in Dr Dale's laboratory, have shown that the  $\text{O}_2$  consumption was increased by this method of artificial respiration. This may have been due in part to an attempt by the tissues to replace  $\text{CO}_2$ . It was very likely due in part to alkalosis since the author has recently found that intravenous injection of  $\text{NaHCO}_3$  in urethanised cats caused a definite increase in  $\text{O}_2$  consumption, whilst intravenous injection of  $\text{HCl}$  produced the opposite effect. The latter phenomena probably constitute a general physiological principle.

experiments and the author hopes to publish the results in the near future.

*CO<sub>2</sub> partial pressure in the tissues and cavities just before and some hours after death.* When an animal was dying the CO<sub>2</sub> tension often rose above 80 mm. just before death. Tables I, II, V and VIII record the high results obtained some hours after death, 193 mm. being obtained in one case.

#### SUMMARY.

1. Injection of air under the skin afforded a ready means to examine changes in CO<sub>2</sub> partial pressure within the body. No anæsthetic was required and the animal was able to move about normally.

2 Intravenous injection of acid reduced the CO<sub>2</sub> partial pressure in air under the skin.

3 Exposure to a warm atmosphere (37° C) greatly increased the CO<sub>2</sub> partial pressure in air under the skin and in the abdominal cavity, although the body temperature was not greatly altered.

4. Exercise markedly increased the CO<sub>2</sub> partial pressure in air under the skin, although the exercise produced only a slight rise of body temperature.

5 Artificial respiration greatly reduced the CO<sub>2</sub> tension in air under the skin and in the abdominal cavity, figures as low as 21 mm being recorded.

The author is much indebted to Dr Dale for the loan of the double-action pump and to Dr Leonard Hill for kindly criticism in the above research.

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## CREATINE FORMATION IN FROG'S MUSCLE CONTRACTED BY NICOTINE.

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INCREASED creatine excretion was found by Pekelharing<sup>(1)</sup> to be produced in man by prolonged continuance in the military position of "attention." For this and other reasons he came to the conclusion that tonic contraction caused a protein breakdown in the muscle accompanied by a formation of creatine. The subsequent experiments have been chiefly on the lines of determining whether the normal tone of muscles and that following decerebration, when diminished by nerve section, does or does not decrease the amount of creatine. In relation to these experiments it has been urged that their electric changes show the contraction to be tetanic and not tonic.

Contraction caused by certain chemical bodies continues for a considerable time and seems to be more nearly a tonic contraction than any that can be produced in vertebrates by nerve stimulation. It seemed then desirable to determine whether this form of contraction is accompanied by creatine formation. The chemical substance the action of which on muscle has been most worked out is nicotine and our experiments have been made with this. The experiments were made on frog's muscle; before operation the cerebral hemispheres were destroyed. It has been shown by Langley<sup>(2)</sup> that nicotine under a certain percentage causes in frog's muscle a primary contraction which in each fibre is confined to the region under the nerve ending—the neural region,—and does not cause an obvious decrease of direct excitability. After a more or less prolonged period contraction begins, and slowly progresses in the rest of the muscle fibre—the general muscle substance; this change is accompanied by a decrease of excitability until the death of the muscle. The action on the general muscle substance begins at once when the percentage of nicotine exceeds a certain value and is rapid with 1 p.c. solution. Our experiments then had a second object, viz. to determine whether the primary, local, and apparently innocent contraction had the same relation to creatine as the general contraction leading to death, or whether the relation was different.

Some experiments with 1 p.c. nicotine were made on frogs by Pekelharing and Hoogenhuyze(3). They injected 1 c.c. of 1 p.c. nicotine dissolved in Ringer into the abdomen of the frog; half-an-hour later on stimulating the sciatic of one side with intermittent electric stimuli they found the greater creatine content on that side. In other experiments the hind limb of one side was placed in 1 p.c. nicotine solution and that of the other side in Ringer, and the sciatic of both sides was stimulated for 30 minutes. In these the creatine content was greater on the side placed in nicotine solution. But in another experiment in which they placed the muscles in 1 p.c. nicotine for 30 mins. without stimulating there was no increase of creatine, and curiously enough they state that they did not notice any rigor at all.

Our first experiments were made on the flexor muscles of the arm in which, as is known, strong contraction is caused by a small concentration of nicotine. The creatine was estimated as creatinine by Folin's method. Analysis of the muscles taken on the two sides from freshly killed unpoisoned frogs showed that the percentage difference in the amount of creatinine on the two sides was slight and variable (*cf.* Table I, A). It varied from 0.96 to 2.7 p.c. but generally was about 1 p.c.

In other frogs, in which the systemic arch (thoracic aorta) was tied on one side above and below the subclavian artery so as to cut off completely the blood supply to the corresponding arm, contraction of the arm muscles on one side only was produced by injecting hypodermically a few drops of 1 p.c. nicotine. The contraction so produced lasts half-an-hour or more and does not greatly affect the excitability of the muscle. It may be taken as being in most cases at any rate a contraction in the neural region. The frogs were killed 25 mins. after the injection and the creatinine of each side was compared. In all the nicotine contracted muscles an appreciable and often large increase of creatinine was found (*cf.* Table I, B). In most of the experiments the increase was about 5 p.c. but it varied from 3.7 to 10.9. It is possible that the higher percentages were due to the general muscle substance being affected.

In discussion on the effect of the sympathetic on creatine formation it has been pointed out that variation of blood supply may cause a variable amount of creatine to be removed from the muscles. The muscles only slowly lose their excitability when the blood supply is cut off and it seemed worth while to determine whether the creatine content is increased or decreased by tying the arteries. In five frogs the systemic arch (thoracic aorta) was tied on one side in the manner mentioned above and after 25 mins. the frogs were killed and the creatinine in the muscles of each

both ends of the sartorius muscle have no nerve-endings. Hence we can roughly divide this muscle into the neural and non-neural region, taking the middle part of the muscle and the cut off ends separately. The sartorii taken from three frogs were thus divided into two regions and each was analysed. From Table IV, A we see that the differences in creatine content of these two parts are insignificant. In the next series of experiments the two regions of the muscle were put separately in a half c.c. of 1 p.c. nicotine and after 10 mins. they were taken for analysis together with the immersing fluid. In the last two of these experiments the intact muscles were first put in nicotine and cut after being taken out. The results (B) show distinctly greater content of creatine in the neural region than in the non-neural region. Similar experiments were also made with .1 p.c. nicotine. Here again (C) the creatine content was found, except in one case, to be greater in the neural than in the non-neural region after 30 mins. The differences were little less than in cases with 1 p.c. nicotine. Although we have above pointed out that the individual variation is so great that the mean value cannot be taken as a standard, we may use it here for the sake of simplicity. From the Tables II and III we get .244 as a mean percentage of creatine in 25 apparently normal sartorii. This is in good accordance with the mean calculated from Table IV, A, i.e. .246. If we compare these mean values with figures in Table IV, B and C, we may again notice that the creatine is more or less increased in the neural as well as in the non-neural region and in each region it is slightly greater in muscles treated with 1 p.c. than those treated with .1 p.c. nicotine. The increase of creatine in the non-neural region when treated with .1 p.c. nicotine does clearly show the discrepancy between mechanical effect and creatine formation caused by nicotine.

Summing up the whole results we may conclude that the tonic contraction of frog's muscle caused by nicotine is always accompanied by creatine formation, though the latter is, to some extent at least, independent of the former.

*Remarks.* The tonic contraction and gradual rigor produced by dilute nicotine are of a type produced either in frog's or in mammalian muscle by a number of chemical substances; curari preventing the primary contraction but not influencing the rigor contraction. Such substances are physostigmine, acetyl-choline, guanidine and sodium sulphocyanide. It is then probable that all these when they cause tonic contraction set free creatine. And it is not improbable that all chemical substances causing tonic contraction set free creatine. With regard to some of these it may be noted that Pekelharing and Hoogenhuyze(?) placed the hind-limb

museles of the frog in solutions of veratrin,  $\text{CaCl}_2$ ,  $\text{NaCyS}$  or caffen and stimulated the seiatic for 30 mins. with induction shoeks 24 times per min. They found increase of creatine in all. They consider that the drugs enabled the nerve stimulation to cause tonic contraction. But since tetanic nerve stimulation in normal conditions has not been found to cause tonic contraction, it seems more likely that the increase in the amount of creatine found by Pekelharing and Hoogenhuyze was due to the contraction either increasing the direct action of the drugs, or to its producing fatigue, and the resulting lactic acid causing the tonic contraction. It may be noticed that the p.c. increase of creatine was in general considerably greater than in any of our experiments.

#### SUMMARY.

Experiments were made on the arm museles and on the sartorii of the frog.

Cessation of circulation slightly increases the amount of creatine in the muscles.

Injection of nicotine causes an appreciable increase of creatine in the flexor muscles of the arm which react most easily to this drug.

In the excised sartorii the increase of creatine differs as the concentration of nicotine and the time of its action differ. Nicotine  $\cdot 1$  p.e.—which causes contraction in the neural region only, continues to set free creatine for about 30 minutes. Since by this time the musele has relaxed, it is concluded that whilst the creatine formation is partly due to the tonic contraction, it is partly due to some further change independent of contraction. Nicotine 1 p.e. which causes rapid rigor and death of the sartorii causes a greater production of creatine than  $\cdot 1$  p.e. but not in proportion to the greater area of musele affected. The maximum amount is formed in about 10 minutes.

Nicotine 1 p.e. as well as  $\cdot 1$  p.e. causes a greater increase of creatine in the neural region of the musele than in the non-neural region, i.e. the greater contraction response of the neural region corresponds with a greater readiness of creatine formation.

In conclusion we wish to express our cordial thanks to Prof. Langley for his kind advice and suggestions throughout. We are also indebted to Prof. Hopkins in whose Laboratory the chemical side of this work was done.

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The Tables give the percentage of creatinine in the muscles. The actual percentage is given on one side. On the other side the deficit or excess of the percentage from that of the first side is given. The last column is the percentage difference of the two sides.

TABLE I. Creatinine in the flexor muscles of the arm

A. Comparison of the two sides in normal frogs

Frog	Right	Diff. left	p.c. diff.
1	.212	-.002	-.05
2	.214	-.004	1.9
3	.323	-.005	1.5
4	.262	+.003	1.1
5 <sup>1</sup>	.229	-.006	2.7
6 <sup>2</sup>	.282	-.003	1.1

<sup>1</sup> Muscles of five frogs taken.  
<sup>2</sup> Muscles of six frogs taken.

B. Injection of 1 p.c. nicotine

	Uncon- tracted	Diff. con- tracted	
1	.246	+.009	3.7
2	.238	+.026	10.9
3	.239	+.010	4.2
4	.232	+.016	6.9
5	.234	+.025	10.7
6	.231	+.012	5.2
7	.230	+.014	6.1
8	.249	+.018	7.2

C. Effect of ligation of artery

	Un- ligated	Diff. ligated	
1	.283	+.009	3.2
2	.334	+.006	1.8
3	.283	+.008	2.8
4	.278	+.007	2.5
5	.292	+.004	1.4

TABLE II. Creatinine in sartorii of three frogs, immersed in .1 p.c. nicotine for:

A. Ten minutes

Exp.	Uncon- tracted	Diff. con- tracted	p.c. diff.
1	.230	+.008	3.5
2	.226	+.004	1.8
3	.243	+.011	4.5
4	.231	+.007	3.0
5	.242	+.011	4.5

B. Thirty minutes

1	.249	+.016	6.4
2	.242	+.021	8.7
3	.241	+.014	5.8
4	.248	+.019	7.7

C. Two hours

1	.259	+.019	7.3
2	.270	+.016	5.9
3	.254	+.017	6.7
4	.254	+.024	9.5
5	.242	+.019	7.9

TABLE III. Creatinine in sartorii of three frogs, immersed in .1 p.c. nicotine for:

A. Five minutes

Exp.	Uncon- tracted	Diff. con- tracted	p.c. diff.
1	.249	+.007	2.8
2	.243	+.016	6.6
3	.243	+.010	4.1

B. Ten minutes

1	.253	+.026	10.3
2	.232	+.023	9.9
3	.246	+.015	6.1
4	.241	+.022	9.1
5	.233	+.020	8.6

C. Thirty minutes

1	.241	+.022	9.1
2	.246	+.018	7.3
3	.245	+.019	7.8

TABLE IV. Percentage creatinine in neural region (mid part) and non-neural region (ends) of muscle. The mid part taken was slightly heavier than the ends

Exp.	A. Normal		p.c. diff.
	Ends	Diff. mid.	
1	.238	+.001	.4
2	.238	+.001	1.7
3	.253	+.005	2.0
4	.246	+.002	.8
5	.248	+.001	.4

B. Placed in 1 p.c. nicotine for ten minutes

1	.249	+.008	3.2
2	.252	+.013	5.2
3	.269	+.011	4.1
4	.259	+.021	8.1
5	.260	+.002	.8

1-3 muscles were cut before placing in nicotine and the solution analysed with them  
 4-5 muscles were cut after placing in nicotine.

C. Placed in .1 p.c. nicotine, the muscles were cut after placing in nicotine

1	.253	+.009	3.6
2	.255	+.012	4.7
3	.257	+.007	2.7
4	.259	+.001	.4
5	.253	+.000	2.4

# ON THE MAXIMUM WORK OF HUMAN MUSCLES ESPECIALLY THE FLEXORS OF THE ELBOW.

By T. E. HANSEN AND J. LINDHARD.

*(From the Laboratory for the Physiology of Gymnastics, University of Copenhagen.)*

As a link in a long chain of excellent works dealing with the physiology of skeletal muscles A. V. Hill<sup>(1)</sup> has recently published a paper on the maximum work of human muscles. The maximum work is obtained when the muscles are loaded in such a manner, that the load in every moment opposes the contraction by a force which the muscle is just able to overcome. This principle may be realized when the muscles contract against the inertia of a heavy fly-wheel such as described in detail by Hill. By the aid of this apparatus Hill has made a series of experiments from which he has drawn interesting conclusions concerning the efficiency of the muscles and their most economical speed. As, however, the results of Hill on certain points need a further investigation, we have thought it worth while by means of a similar apparatus to make some additional experiments the result of which we propose to discuss in the present paper.

Our fly-wheel (Fig. 1) consists like that of Hill of a number of pulleys on which the string may be wound up, but moreover it has an iron disc furnished with a heavy ring of lead, just like the fly-wheel in the bicycle-ergometer of Krogh. We obtain in this way a very large moment of inertia. The wheel is mounted on an iron frame furnished with ball-bearings for the shaft. Near the end, the shaft has a worm and wheel transmission to move a small index rotating on a horizontal disc provided with a mark indicating every twenty revolutions of the fly-wheel. This counting device has so little resistance, that it has no appreciable influence on the speed of the wheel. The wheel is stopped by means of a wooden brake acting upon the lead-ring<sup>1</sup>.

Owing to the large moment of inertia of the wheel we have had some trouble to find a suitable string strong enough to stand a heavy pull and on the other hand flexible enough to offer no resistance when wound

<sup>1</sup> The fly-wheel has been built in the workshop of the zoophysiological laboratory of Copenhagen (Prof. Krogh).

round the smallest pulley. At first we used a violoncello string but later we adopted a very thin steel band. The wheel is calibrated in the manner

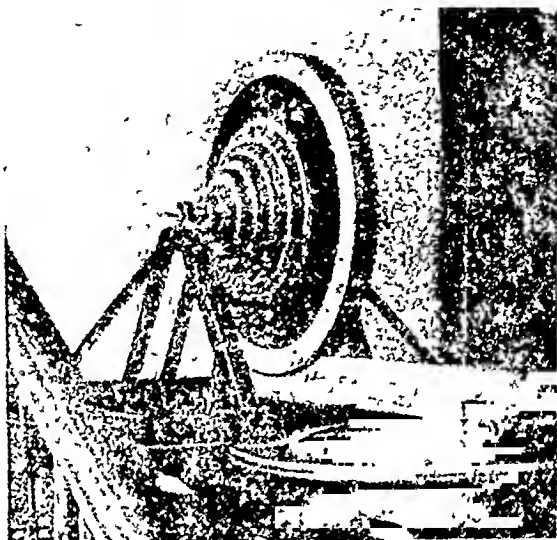


Fig. 1.

described by Hill and the moment of inertia has been determined to  $(.828 \pm .0029) \times 10^7$  expressed in absolute units. The "equivalent masses" corresponding to the seven pulleys are respectively: 60, 87, 134, 235, 507, 1197, 5849 kilograms, and the work done in a pull is  $W = 1.666 \times r^2$  kilogram-metres when  $r$  is the number of revolutions per second.

Our experiments proceeded in the manner described by Hill and regarding the relation between work and "equivalent mass" we obtained quite similar results. From the curve representing this last named relation Hill determines the theoretical maximum work  $W_0$ . This figure, which is of the greatest interest for all the deductions in Hill's paper, must, if possible, be determined exactly. The author is of opinion, that  $W_0$  is determinable from the curves with a limiting error of about 1 p.c. We do not believe, however, that this is possible and have therefore endeavoured to determine  $W_0$  independently of the curves.  $W_0$  may be expressed mathematically by the equation  $W_0 = \int T dl$  when  $T$  is the maximum tension of the string and  $l$  the length of the pull in the direction of the string, thus  $W_0$  is represented by the area of a curve the ordinates of which are  $T$  and the abscissa of which is the length of the pull. This area can be measured by means of a planimeter and we are thus able to

determine  $W_0$  with considerable accuracy if we can only determine  $T$  in a definite number of points and the length of the pull. To determine  $T$  we fix the string around the smallest pulley of the wheel and between this and the handle we insert a Collin's dynamometer. The scale for pull on the dynamometer may be very incorrect and the apparatus must therefore be carefully calibrated before use. Apart from this the dynamometer is a very suitable instrument for the present purpose as the change of form caused by the pull exercised by a man's arm is negligible, the pull being thus really isometric. When doing an experiment the subject places his arm on the table in the usual manner and the length of the string being successively varied makes a series of maximal pulls against the dynamometer. The corresponding positions of the handle have been determined graphically. When the height of the axis of the elbow-joint and that of the axis of the wheel above the table on which the arm is resting and the horizontal distance between the two axes are known and when the length of the forearm (i.e. the distance from the axis of the elbow to the centre of the handle) and the height of the centre of the handle are determined in each position the whole movement may be figured graphically in natural size on a sheet of paper. During the pull the wrist-joint is always somewhat bent (see Fig. 1 in Hill's paper), and this flexion increases a little as the flexion in the elbow proceeds, thus the length of the forearm as defined above decreases to a corresponding extent and the curve described by the centre of the handle around the axis of the elbow-joint is not exactly a circle. If in addition the angle between the line from the centre of the elbow-joint to the centre of the handle (the mechanical axis of the forearm) and the anatomical axis of the forearm is measured, the angles of flexion of the elbow-joint corresponding to the points in which the maximal tension of the string has been determined may be constructed graphically on the sheet. Of course measurements like these cannot be absolutely exact, but we feel sure that the unavoidable errors do not vitiate the results obtained in any serious manner.

We have in the manner described determined  $W_0$  in a number of experimental series on two male subjects, each series beginning at the smallest angle of flexion and going to maximal flexion and back again. In corresponding positions the average of the dynamometer readings were taken; the angle of flexion of the elbow is reckoned from the table to the anatomical axis of the forearm. The "length of the pull" has been found by projecting the curve described by the handle on the various directions of the string as determined in the points mentioned above. The areas determined by the tension curves and the coordinate axes represent the theoretical



TABLE I.

E. H.	$t$	$W$	$W_0 - W$	$k$	J. L.	$t$	$W$	$W_0 - W$	$k$
$W_0 = 14.1$	.5	7.40	6.70	3.35	$W_0 = 15.3$	.54	7.77	7.53	4.06
	.625	7.75	6.35	3.97		.62	8.46	6.84	4.24
	.74	8.63	5.47	4.04		.80	9.18	6.12	4.89
	.97	9.82	4.28	4.14		1.07	10.41	4.89	5.23
	1.475	10.41	3.69	5.45		1.5	11.37	3.93	5.90
	2.17	10.96	3.14	6.82		2.4	12.50	2.80	6.72
	5.03	11.54	2.56	12.88		5.15	13.95	1.35	6.96

The table shows, that  $k$  increases with increasing values of  $t$ . The two subjects behave in quite the same manner though quantitatively different, and the systematic character of the variation excludes possible errors on the time-determination as the cause. On the other hand it must be admitted, that the determination of  $W$  involves an error which tends towards too low values for  $k$  in experiments on the larger pulleys. If we raise the table against the axis of the wheel about twice the radius of the largest pulley,  $W_0$  in the subject J. L. increases by .8 kilogram-metre. Assuming  $W_0$  in a pull on this pulley to be 15.7 instead of 15.3,  $W_0 - W$  increases also by .4 kilogram-metre, and thus  $k$  will increase by about .2 or 5 p.c. The difference between  $k_7$  and  $k_1$  is however 1.67 or about 43 p.c., and we do not doubt, therefore, that the increase in  $k$  is real. In the subject E. H. a rise in  $k$  due to the cause here concerned would only amount to 2.7 p.c., the difference between  $k_7$  and  $k_1$  being 284 p.c. It may be added, that the variation in the values of  $k$  shows itself in each single series of experiments, though in a less regular manner. It is most probable, that this fact has been overlooked by Hill, because his "equivalent masses" on the whole are remarkably smaller than ours, and it is only corresponding to the greatest "equivalent masses," that  $k$  as a rule is quite out of proportion. The real cause may be, that Hill's  $W_0$  does not represent the theoretical maximum work but is a rather arbitrary figure as will be shown below.

This point of view has been confirmed by a quite recent paper of Lupton(2). The author has introduced some technical improvements in Hill's apparatus and has examined a number of subjects. His results agree with those of Hill. Lupton gives two tables from which we can calculate  $k$ . Table IV gives constant values, but when calculating  $k$  from Table V we find an unmistakable variation, though the value is comparatively low corresponding to the largest "equivalent mass." If we suppose, however, that  $W_0$  in Table IV is increased by 1 kgm. and calculate  $k$  from this supposition we find values varying regularly from 3.41 to 4.88. If we further, for the subject E. H. in our Table I, suppose  $W_0$  to be 13.1 instead of 14.1 we find a fairly constant value for  $k$ . It is thus evident,

that the determination of  $W_0$  is the turning point. And we do not doubt, that the  $W_0$  of Hill and of Lupton is too low. The least difference  $W_0 - W$  is in the two tables of Lupton 1.26 and 1.23 respectively, but in our Table I the difference between  $W$  on the "equivalent mass" corresponding nearly to the largest "equivalent mass" of Hill and on our largest "equivalent mass" is 1.13 and 2.58 respectively, and it is at least very probable, that other subjects would reach quite similar values for  $W$  if the "equivalent masses" pulled on were only large enough. We have in the above always expressed  $k$  in arbitrary terms but it is evident, that a translation into absolute units does not alter the relation.

Hill regards  $k$  as a constant depending upon the coefficient of viscosity of the muscle and varying with its mass. Regarding our method for determining  $W_0$  as more reliable than that of Hill we must hold, that  $k$  is not a constant but a variable including probably a constant viscosity-factor. The two points of view might seem to be in serious opposition; we believe, however, that the discrepancies are only small and propose the following explanation.

The cause of the systematic variation in  $k$  must be sought in  $W$  which is related to time not only through the variations in the link  $k/t$  in Hill's equation. We must assume, that the maximal tension can only be maintained for a fraction of a second. The whole problem is rather complicated and cannot be finally solved at present, but we will try to clear up the outlines of the question. First it must be remembered, that the term maximum tension cannot be exactly defined *a priori*. If the "all-or-none" principle is valid also for skeletal muscles which we will assume at least so far as the tension is concerned, then the maximum tension is obtained when all the individual fibres are stimulated simultaneously. This may be possible when experimenting on the isolated muscle, though no one knows if a maximal stimulus really excites all fibres or only "the largest possible number." As for human muscles *in situ* when an artificial stimulus is applied it is very improbable, that all the fibres in a muscle are caused to contract simultaneously, because the strong electrical stimuli give rise to severe pain in the skin, and we possess no means of ascertaining whether all fibres contract or only a fraction of the whole number. On the other hand every one who has experience of having his muscles stimulated by electrical stimuli knows, that it is possible in this way to obtain contractions of a force which it is quite impossible to reproduce voluntarily. It is likewise a well-known fact, that lunatics or patients suffering from convulsions or tetanus may develop a muscular force quite out of proportion to what the same persons are able to yield when normal. Therefore, when a muscle

on voluntarily innervated muscles the "fatigue factor" cannot be omitted, it is just as physiological and just as unavoidable as is the "viscosity factor." The sort of "fatigue" here concerned does not show itself on the work done on the large "equivalent masses" only but may be traced backwards also to the work against the small "equivalent masses." The other alternative, that  $W_0$  might be defined in such a manner, that allowance was made for the "fatigue factor," is likewise out of question, theoretically, because  $W_0$  then would be not a constant but a variable, practically, because it would claim an overwhelming work to determine a series of values of  $W_0$  related to different values of  $t$  and  $W$ .  $W_0$  as determined by Hill only corresponds to a certain definite value of  $t$ , for smaller values of  $t$  it would have a greater value. The equation of Hill is not erroneous but it is incomplete, making no allowance for a factor which is unavoidably related to voluntary contraction.

This being so the efficiency of the work cannot be calculated as done by Hill. We have therefore endeavoured to find out the efficiency by determining the increase in oxygen consumption due to the work, but unfortunately also this way is impracticable. The "static work" necessary to maintain the position of the body during a heavy pull is so overwhelming, that the efficiency of the work done in the pull calculated from these data only amounts to a small percentage. The experiments have been carried out by means of Douglas-bags. The base-line was determined in a five minute experiment on the standing, resting subject. Then in the moment when the pull began the tap was turned to a new bag and an experiment lasting 5 or 6 minutes was performed; after this experiment the tap was turned directly to a third bag and a third experiment lasting 4 to 5 minutes begun. During the work the subject always holds his breath, thus the whole increase in the gas exchange takes place after cessation of work. Table II shows the results.

TABLE II.

Subject	"Equiv. mass."	Work		Excess of O <sub>2</sub>		Efficiency per cent.
		kgm.	Cal.	c.c.	Cal.	
E. H.	60	7.4	-0174	143	-704	2.5
	235	9.8	-0230	198	-975	2.4
	5849	11.55	-0270	340	1.68	1.6
J. L.	60	7.8	-0183	137	-656	2.8
	235	10.4	-0244	212	1.020	2.4
	5849	13.95	-0326	406	1.950	1.7

The oxygen consumption reaches the resting level within 5 to 6 minutes. Assuming the efficiency of the work on the "eq. mass" 235 (duration of the pull about 1 second) to be 25 p.c. the total heat production,

H, in the case of E. H. should amount to .092 cal. or about 19 c.e. of oxygen. Thus the "static work" demands .883 cal. or, the efficiency of maximum "static work" being some 50 p.c., it amounts to 189 kgm. or nearly twenty times the work done on the wheel. These experiments seem to show, that the only way to obtain reliable figures for the efficiency of human muscles is the determination of the oxygen consumption in working experiments, where the "static work" is insignificant as compared with the dynamic work. This demand may be fulfilled by work on the bicycle-ergometer.

Regarding the flexors of the elbow Hill is not rightly informed by Prof. Stopford. This group of muscles has been very thoroughly investigated by Braune and Fischer(3) and in our opinion it would require very strong reasons to discard their experimental results. The muscles in question are: Mm. brachialis internus, biceps brachii, brachio-radialis, extensor carpi radialis longus and pronator teres. These muscles are at work when the elbow-joint is bent and other muscles have no appreciable influence on this movement. Other muscles may however influence the position of the elbow on the table and thus indirectly the pull on the string. It is therefore a sufficient and necessary condition to get a pure action of the flexors of the elbow on the string, that the arm and shoulder are fixed on the table; when this condition is fulfilled it is quite impossible for other muscles, *e.g.* the pectoralis, to act on the string, and the position of head and neck, trunk or legs is absolutely irrelevant. As, however, it may be difficult in the beginning to keep the arm quiet owing to the great number of muscles put in static action to secure the stability of the body during the pull, it will be preferable, as done by Hill, to order the subject always to maintain a definite position during the experiment.

From the work diagram (see p. 291) combined with the angles of flexion of the elbow-joint as measured on the graph, the shortening of the muscles as given in the tables of Braune and Fischer and the relative cross-sections of the muscles concerned we are able to calculate the maximum tension of the muscles and its variation with the angle of flexion. Such a calculation may claim some interest, as the tension of human muscles and its variation with the shortening of the muscles has hitherto been a matter of discussion. The tensions are given corresponding to the mean of the intervals between the points fixed when determining  $W_0$ . The shortening of the muscles in these intervals is interpolated from the average table of Braune and Fischer. We believe, that these values may be used in a concrete case without any serious error. On the other hand the cross-sections or the masses of the muscles vary so enormously according to the

When we have chosen to give the actual tensions of the muscles and not to consider their cross-sections the reason is not only the great variations found in the anatomical data and the impossibility to obtain any measurement in a concrete case, but we must, according to our opinion set forward above, consider the maximum tension as an index of the innervating power of the subject. The maximum tension on the unit of cross-section of one or other human muscle stimulated with artificial maximum stimuli cannot be supposed to vary to any great extent; on the other hand the tension actually found by voluntary maximum stimulation may vary very much in different subjects, and it may vary in the same subject from day to day under the influence of training or fatigue. The fact, that the maximum work done in a very plain movement increases conspicuously from one day to another, is indeed a strong support to our view.

#### SUMMARY.

The theoretical maximum work of the flexors of the elbow has been determined. The maximum tension of these muscles and its variation with the angle of flexion of the joint is given.

It is shown, that Hill's equation,  $W = W_0 - k/t$ , is not valid in its present form for voluntarily stimulated muscles.

For valuable advice we are indebted to J. P. Möller, B.Sc.

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## CALCIUM CHLORIDE ACIDOSIS.

By J B S HALDANE, R HILL, AND J M LUCK

*(From the Biochemical Laboratory, Cambridge)*

GYORGI(1) has shown that calcium chloride, when administered to babies, causes an increased ammonia and acid output in the urine. The experiment here described was made on J B S H (100 kilos) and lasted for 13 days, during three of which he drank 85 grams (766 gram molecule) of calcium chloride in 3.5 litres of water. The food intake was approximately constant through the experiment. The water intake, where above normal, is shown in the table.

Alveolar carbon dioxide was estimated by the Haldane Priestley method, each daily value being the mean of four pairs of samples. In the urine pH was estimated with Clark-Lubs indicators and buffer mixtures, acid excretion by titration to pH 9 with 1 N soda and thymol blue, buffer excretion by titrating back to pH 3.7 with 1 N hydrochloric acid and thymol blue. A small correction, rarely exceeding 1 p.c., was made for the acid uncombined at the latter reaction. Ammonia was estimated by van Slyke's(2) method, phosphates with uranium acetate, chloride by Volhard's, and calcium by McCrudden's(3) method. A Colc-Onslow(4) comparator was used when necessary. Faecal carbonate was estimated as carbon dioxide in J S Haldane's(5) blood-gas apparatus, faecal phosphorus with uranium acetate after boiling with nitric and sulphuric acids.

In order to bring 0.5 N ammonium chloride solution to pH 9 it was found necessary to add 16 p.c. of its equivalent of 1 N soda, the salt being partly hydrolysed, while on subsequent addition of formaldehyde only 85 p.c. of its equivalent was needed to bring it back to pH 9. Hence if we add the ammonia and acid excretions found in this research, we obtain a value for the total acid eliminated free and in combination with ammonia which is too high by 16 p.c. of the ammonia value. 16 p.c. of the ammonia values have therefore been subtracted from the acid excretion, and the result given as "net acid." If the ammonia is titrated with formaldehyde the total value is correct, except for the small error due to amino acids, but the ammonia values are too low. The buffering

between pH 9 and 3·7 is due in part to the hydrolysed fraction of the ammonia, in part to phosphates, each molecule of which buffers one equivalent of acid. The buffer excretions when these values have been deducted are given as "undetermined buffers." Urines were collected in 12 hour periods from 9.30 to 9.30. Quantities ingested and excreted are given in milligram-molecules, or c.c. *N* solution per 12 hrs.

Calcium chloride in 3 p.c. solution produced no nausea, but there was intense diarrhoea, followed by constipation due to the formation of a large hard faecal mass. There was great general discomfort, pains in the head, limbs and back, and disturbed nights. These effects never occur with ammonium chloride, and must be attributed to the calcium. The alveolar carbon dioxide fell, while acid, ammonia, and phosphate excretion rose, their relations being similar to those found by Haldane(6) in ammonium chloride intoxication and leaving no doubt that an acidosis is produced. The *cH* soon reached its maximum, followed by acid and phosphate excretion, and slightly later by ammonia and the carbon dioxide minimum. The ammonia excretion was still four times normal when the acid and phosphate had returned to their usual values. This was due to a depletion of the phosphate available for excretion, whilst there were still sources for ammonia. The same cause accounts for the exaggerated diurnal drop in phosphate excretion. The pH fell to a fairly steady minimum value of 4·9 comparable to that of 4·7 recorded by Henderson and Palmer(7). It is probably analogous to the maximal concentrations of urea, chloride, and bicarbonate found by Ambard and Papin(8) and Davies, Haldane and Peskett(9). Its early fall may betoken renal fatigue. There was no evidence of a maximal concentration of any other constituent being reached, though on the night of the 20th-21st the ammonia reached ·250 *N* and the acidity ·131 *N*. The buffers varied greatly, the undetermined buffers much less, the extreme values for daily excretion being 75·9 and 57·4, though the results of the 25th suggest that they can be washed out by drinking. Their relative steadiness is intelligible since the most important weak electrolytes in urine unanalysed here are uric acid and creatinine. The phosphates on the average only contributed one-third of the total buffering power, while the ammonia occasionally contributed more than the phosphates.

The calcium excretion fell to nearly normal figures within 12 hours of the last salt being taken. The extra calcium in the urine before this time accounts for only 9·3 p.c. of that ingested. The small increase in the calcium excretion which remained is probably to be attributed to

the acidosis, as in the experiments of Givens and Mendel(10). The chloride figures show that 1.41 gram molecule above normal was excreted within 12 hours of the last salt being taken, out of 1.43 ingested, so the chloride must have been completely absorbed.

The negative balance of urinary phosphorus amounted to 85.8 millimols, or 2.66 grams on Oct. 21st, but taking the experiment as a whole it was only .37 gram, which may well be due to irregularity of the diet. There can therefore have been little increased loss of phosphorus in the faeces. It was impossible to collect these completely, but a stool of Oct. 19th contained 11.2 p.c. of solids, which gave off 71 c.c. of carbon dioxide at 0° and 760 mm. per gram on the addition of acid. This corresponds to a calcium carbonate content of 31.6 p.c. If all the faecal phosphorus found was present as calcium phosphate ( $\text{Ca}_3\text{P}_2\text{O}_8$ ), (and according to Rogozinski(11) about half of it is organic) the calcium phosphate only amounted to 9.3 p.c. of the dry weight. So at least 3.5 times as much calcium was being excreted in combination with carbonic as with phosphoric acid. A sample of normal faeces contained only .16 p.c. of calcium carbonate, even if all the combined carbon dioxide was in this form. No hydrogen sulphide was evolved on adding acid. There can be no doubt that calcium carbonate excretion was the main cause of the acidosis. In the neutral intestinal contents calcium carbonate was precipitated, bringing down the carbonate of the digestive juices and replacing it by chloride which was re-absorbed in accordance with the equation



Thus, as has been shown to be the case by Baird, Douglas, Haldane and Priestley(12) in ammonium chloride acidosis, the  $\text{HCO}_3$  of the plasma and tissues is partly replaced by  $\text{Cl}$ , the alveolar carbon dioxide falling simultaneously so as to keep the  $\text{cH}$  of the blood approximately constant.

The alveolar carbon dioxide with a normal acid and ammonia excretion was 34–35 mm. as against 39–40 mm. in 1920, and 37–38 in the springs of 1922 and 1923. There was no evidence of kidney disease, and we are unable to account for the changes. The ammonia + acid excretion showed a roughly linear relation with the alveolar  $\text{CO}_2$  as in 1920, but in this case the kidneys were somewhat more responsive, excreting as much acid + ammonia at low alveolar  $\text{CO}_2$  pressures, and less at high ones.

Mr R. Webb, of the Pathological Laboratory, very kindly estimated



the hæmoglobin of the blood on Oct. 19, 20, and 23, and performed cell-counts on the first two dates. In addition, the hæmoglobin and cells were determined after the close of the experiment. The hæmoglobins were 12·5, 12·5 and 10 p.c. above normal, while the red blood corpuscles were increased by 10 and 8 p.c., the whites by 31 and 30 p.c., the increase in lymphocytes being especially marked. There was thus a concentration of the blood as found by Baird *et al.* (12) with ammonium chloride.

Calcium chloride has been successfully used by Blum (13) and his pupils in the treatment of dropsy, œdema and exudations. They find that large oral doses produce diuresis which may outlast the treatment for days, and great loss of sodium. The plasma proteins become more concentrated, and the weight falls. During calcium chloride ingestion in this experiment there was a large loss of water, partly by diarrhœa, and of sodium or potassium since the chloride excretion increased very much more than that of ammonia. Later there was retention of water, accompanied by thirst from Oct. 21st to 24th inclusive. There was also very great sodium or potassium retention, the ammonia excreted being sometimes enough to neutralize all the phosphoric and hydrochloric acids. Blum attributes the loss of sodium and water to antagonism between sodium and calcium, but this cannot be correct since the effect of ammonium chloride is precisely similar. In both cases  $\text{HCO}_3'$  is replaced by  $\text{Cl}'$  in the blood and tissues. But so great is the loss of salt and water that the total amount of chlorine in the body remains unaltered. This appears from the urine analyses and also from the following observation. During a severe ammonium chloride acidosis 40 grams of sodium bicarbonate were eaten with water in two hours. This would normally cause diuresis and bicarbonate excretion. But neither took place nor did any extra chlorides appear in the urine. All this salt was therefore retained, the change of reaction making this possible.

A possible explanation of these phenomena is as follows. The reaction of the arterial plasma is kept nearly constant by the respiratory centre, but that of the tissues and capillary blood becomes more acid owing to loss of buffering power. For the excess of  $\text{CO}_2$  pressure in the tissues over that in the blood, and the excess of  $\text{CO}_2$  content of venous over arterial blood presumably remain unaltered, and therefore form larger proportions of the total  $\text{CO}_2$ . The increase in hæmoglobin and other protein content is not enough to counteract this effect. The increased acidity brings the blood and tissue proteins nearer to their isoelectric points, and they therefore release cations which they are holding in

Donnan equilibrium, and diminish their osmotic pressure, thus losing water. If this view is correct, ammonium chloride or phosphate should prove a valuable alternative to calcium chloride where the latter is indicated as a diuretic.

## SUMMARY.

1. Calcium chloride taken orally is mainly excreted in the faeces as calcium carbonate, the  $\text{Cl}^-$  replacing  $\text{HCO}_3^-$  in the body and causing acidosis.

2. In calcium chloride and ammonium chloride acidosis the body loses water and salts.

3. Phosphates and ammonia account for less than half the buffering of normal urine between  $\text{pH}$  3.7 and 9.0, but for more than half during acidosis.

TABLE

Date	$\text{CaCl}_2$	Extra	Alv $\text{CO}_2$	Vol	pH	$\text{NH}_3$	Acid	Net	Phos	Buffers	In	$\text{Cl}^-$	$\text{Cl}^-$
Oct	taken	water	mm Hg	cc				acid	phate		detec-		
		drunk									ted		
		c.c.									in med-		
											icuffs		
16	—	0	34.4	1005	6.4	24.2	34.2	31.3	19.0	01.2	37.4	2.8	162
16-17	—	—	—	453	6.9-	22.2	30.9	33.3	10.9	55.0	31.5	1.7	39
17	225	1500	32.2	2815	5.6-	78.0	56.3	33.6	24.8	71.5	34.2	10.4	443
17-18	—	—	—	1175	4.9	57.3	62.2	53.0	25.7	70.3	35.4	9.6	129
18	275	1000	30.3	1620	4.9	64.2	70.0	53.9	31.8	76.5	34.4	14.0	365
18-19	—	—	—	1450	5.0-	99.9	85.7	69.7	34.7	88.3	37.0	16.5	333
19	275	1000	27.3	1475	4.9+	102.4	85.7	60.3	36.1	91.5	39.0	16.2	363
19-20	—	—	—	1705	5.0+	133.2	93.8	72.5	37.3	95.5	36.0	17.1	381
20	—	0	25.4	880	5.2-	139.3	78.2	55.9	29.5	88.6	36.8	5.5	189
20-21	—	—	—	480	5.5+	119.9	02.9	43.7	25.9	78.3	33.2	4.2	75
21	—	600	28.0	600	5.4	128.3	54.0	34.5	17.0	67.3	29.7	4.7	125
21-22	—	—	—	438	5.6-	105.0	50.0	33.2	17.8	62.5	27.9	3.0	71
22	—	600	32.9	440	5.6	90.9	38.0	23.6	8.2	52.8	30.1	3.7	93
22-23	—	—	—	445	5.7-	71.4	38.0	20.6	11.9	55.3	32.0	—	64
23	—	600	34.9	1092	5.9	70.1	31.2	20.0	6.3	47.1	29.6	—	105
23-24	—	—	—	581	0.0	42.1	30.0	23.9	14.6	48.0	27.6	—	81
24	—	400	35.0	1258	6.4+	54.3	27.2	18.5	6.0	43.7	28.4	—	—
24-25	—	—	—	560	6.1	37.4	24.8	18.8	15.0	50.0	29.0	—	—
25	—	ad lib.	34.7	1980	6.4	49.9	28.5	20.6	6.8	61.2	46.4	—	—
25-26	—	—	—	502	—	25.0	26.3	22.3	19.2	47.8	24.6	—	—
26	—	ad lib.	33.9	2020	—	42.8	24.8	18.0	10.3	54.7	37.6	—	—
26-27	—	—	—	473	—	22.8	31.6	28.0	20.3	52.3	29.3	—	—
27	—	ad lib.	33.9	1464	—	31.7	21.8	16.7	12.3	54.6	37.3	—	—
27-28	—	—	—	472	—	—	—	—	19.6	—	—	—	—
28	—	ad lib.	—	2030	—	—	—	—	13.1	—	—	—	—
28-29	—	—	—	343	—	—	—	—	20.9	—	—	—	—

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# THE SYMPATHETIC INNERVATION AND THE PROCESS OF NORMAL SALIVARY SECRETION<sup>1</sup>.

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IN the works of Heidenhain<sup>(1)</sup>, Langley<sup>(2)</sup>, Carlson, Greer and Becht<sup>(3)</sup>, Carlson and McLean<sup>(4)</sup>, and Babkin<sup>(5)</sup> no special importance is given to the secretory fibres of the sympathetic nerve. In this paper I give an account of experiments on the relation of the sympathetic to the reflex secretion of saliva.

My investigations were performed on a dog with permanent fistulas of mixed glands (submaxillary and sublingual glands) and the parotid gland on the left side. On April 15th the dog was anaesthetised; a considerable piece of the left lingual nerve was cut out with the chorda tympani, so that the mixed glands were innervated by the sympathetic nerves and by these only. On the day after the operation the dog was fed with a mixture of meat and biscuit powder. Immediately saliva flowed out of the duct of the parotid, but not a drop came from the ducts of the mixed glands, i.e. the results were the same as those observed long ago on acute as well as on chronic experiments by all who have studied reflexes of the mouth area. The next day and on the fourth day, I again fed the dog, and again not a drop of saliva flowed out. Then 1 c.c. of .25 p.c. solution of pilocarpine was injected subcutaneously, i.e. a total of 2.5 mgr. After a short interval saliva began to flow and the quantity of saliva was measured every five minutes. The secretion of saliva, after first increasing, began to decrease. I then fed the dog several times during five minutes with meat and biscuit powder. The mixed glands, although the chorda was cut, secreted a visibly greater amount of saliva during the time the dog was fed. Evidently the impulses of the mouth area reaching the salivary centre were transmitted to the gland by the sympathetic nerve. The amount of saliva after 0.8 c.c. increased during the feeding to 1.3 c.c.

Ap. 19. 1 c.c. 0.25 p.c. pilocarpin solution was injected under the skin. Saliva in successive 5 minutes (mixed glands): 0, 0.7, 1.0, 1.3, 1.2, 0.9, 0.8, 0.8, 1.3, 0.8, 0.4, 0.3, 0.3, 0.2.

<sup>1</sup> The fundamental facts of this study have been reported in the meetings of the Medical Society at the Odessa University, May 27th, 1919.

<sup>2</sup> Fed with meat and biscuit powder.

I may mention that if the animal is not fed the curve of the salivary secretion under pilocarpine from the denervated gland, after beginning to fall, never rises. The following experiment shows the effect of pilocarpine alone.

Ap. 28. 1 c.c. of .25 p.c. solution of pilocarpine injected under the skin. The amount of saliva was recorded every 5 minutes.

Mix. gl. 0, 1.2, 1.8, 1.8, 1.9, 1.8, 1.4, 1.4, 1.2, 0.9, 0.8, 0.7, 0.5, 0.4.

Parotid 0, 0.5, 0.8, 0.75, 0.7, 0.8, 0.55, 0.6, 0.4, 0.4, 0.2, 0.4, 0.2, 0.3.

When during the "pilocarpine" secretion the dog was fed or had a .25 p.c. solution of hydrochloric acid poured into its mouth, the results were as follows:

Ap. 22. Mix. gl. 0, 2.1, 4.6, 4.8, 4.0, 3.9, 6.0<sup>1</sup>, 2.7, 1.8, 3.1<sup>1</sup>, 2.0.

Parotid 0, 0.6, 1.3, 1.3, 1.2, 1.0, 2.7, 0.8, 0.5, 2.9, 0.4.

M. g. 1.6, 1.2, 0.9.

P. 0.35, 0.3, 0.2.

<sup>1</sup> Fed with meat and biscuit powder.

Mix. gl. 0, 1.5, 3.7, 3.7, 3.1, 3.5<sup>1</sup>, 2.1, 1.7<sup>2</sup>, 2.5<sup>3</sup>, 1.3, 0.9.

Parotid 0, 0.8, 1.5, 1.1, 1.2, 3.1, 0.8, 0.8, 2.9, 1.0, 0.1.

M. g. 1.1<sup>1</sup>, 0.4, 0.3, 0.2, 0.2, 0.3<sup>4</sup>.

Par. 4.3, 0.05, 0.2, 0.05, 0.05, 1.7.

<sup>1</sup> Hydrochloric acid. <sup>2</sup> Water. <sup>3</sup> Meat and biscuit powder. <sup>4</sup> Bread.

The increase of the quantity of saliva during the feeding or during the infusion of hydrochloric acid is very distinctly shown in these experiments. But on pouring water into the mouth although the dog moved its jaws and swallowed the water, the amount of saliva decreased as if nothing had been given. Consequently there was no squeezing out of the secretion during mastication and swallowing.

In order to make certain that a real reflex of the mouth area through the sympathetic nerve has been exhibited here, I cut the sympathetic nerve whilst the pilocarpine salivary secretion was going on. On April 27th the dog was anæsthetised with æther and chloroform, but without morphia. The left vago-sympathetic was isolated and a thread passed underneath it; the ends of this were placed under the skin to enable the nerve to be pulled out of the wound, and the skin was sutured. The next day the dog showed no ill effects of the operation. On feeding the dog with meat and biscuit powder the usual effect of increase of the outflow was produced. The vago-sympathetic nerve was then lifted on the thread and cut. The dog endured the operation quite calmly and during the first five minutes immediately after, it ate as willingly as before. The secretion in each five minutes after giving pilocarpine was as follows:

Ap. 28. Mix. gl. 0.2, 2.3, 3.4, 3.4, 3.9, 3.45, 6.0<sup>1</sup>, 2.1<sup>2</sup>, 1.4<sup>1</sup>.

Parotid 0.07, 1.0, 1.1, 1.2, 1.1, 0.9, 2.8, 0.4, 2.0.

M. g. 1.4, 1.4, 1.4, 1.4, 1.3<sup>1</sup>, 1.1, 0.9.

Par. 0.2, 0.3, 0.5, 0.4, 1.9, 0.3, 0.05.

<sup>1</sup> Meat and biscuit powder.

<sup>2</sup> N. Vago-sympathetic cut.

This experiment shows that the sympathetic nerve participates in the transmission of the reflexes from the mouth area. Before the secretion of the sympathetic feeding caused the quantity of saliva to rise from 3.45 c.c. to 6.0 c.c.; and section of the sympathetic nerve put an end to any increase of secretion.

Anrep(7) has attributed the augmented secretion to a contraction of the ducts. This explanation does not satisfactorily account for the increased secretion described here, since in some of the experiments (Exp. Ap. 19) there was no decrease in the secretion in the five minutes following feeding and there should be a decrease if the ducts contracted. I conclude that the impulses set up in the sympathetic by feeding pass in part at any rate by secretory nerve fibres. In the course of following months the dog was repeatedly fed during the pilocarpine secretion, but always without any influence on the salivary secretion. But the parotid gland, the cranial nerve to which was intact, continued to respond to stimulations in the mouth area, by reflex salivary secretion.

In this experiment (Ap. 28) the pilocarpine salivary secretion from the mixed glands remained at about the same rate for 25 minutes. I did not find this either in the preceding or in the following experiments. A possible reason for the maintenance of the rate may have been a continued dilation of the gland vessels such as has been noted by Burton-Opitz(8) in acute experiments after the section of the sympathetic nerve. It is also possible that there was some stimulation proceeding from the cut end of the nerve. I may add, that the absence of marked increase of salivary flow in the above case, and merely its prolongation, speaks to a certain degree against the vascular origin of the increased secretion of the pilocarpinised salivary gland during the excitation of the mouth. After the section of the vago-sympathetic, the diminution of the tone of blood vessels of the salivary gland was maximal. Nevertheless this dilation of salivary blood vessels did not increase the secretion to the same degree as the feeding of the animal or pouring into the mouth of hydrochloric acid. This question ought, however, to be especially investigated.

I calculated the increase of pilocarpine secretion after introducing into the mouth some meat powder or hydrochloric acid. The average figures were as follows: When meat powder or meat and biscuit powder was given the increase was 1.61 times in 5 minutes (average from 6 experiments), but when the acid was poured in 1.12. In reality the increase is greater, because in the calculation no account is taken of the normal decrease in rate of secretion.

I may mention that feeding with bread caused similarly less secretion

than feeding with meat and biscuit powder. The fact that reflex sympathetic secretion is produced in unequal quantities by different foods shows, I consider, that the sympathetic can conduct secretory impulses of different kinds. This conclusion is in harmony with that of Babkin (6) with regard to the chorda tympani.

A few words may be added about the parotid of the same dog. My experiments on this gland were few, but so far as they went it seemed clear that section of the sympathetic decreased the amount of saliva caused by a given stimulus. The absolute increase is, of course, greater the less the rate of secretion before feeding. In one experiment the secretion before feeding was 0.1 c.c. in 5 minutes and feeding increased the secretion to 4.3 c.c.—*i.e.* 43 times. After cutting the sympathetic the secretion before feeding was also 0.1 c.c. but feeding only increased it to 1.3 c.c., *i.e.* 13 times. In the Exp. of April 28th feeding had previously increased the secretion from 0.5 c.c. to 2.9 c.c., *i.e.* 5.8 times. On April 28th after cutting the sympathetic feeding only increased the secretion from 0.4 c.c. to 1.9 c.c., *i.e.* 4.7 times.

The fact of the transmission of the reflex from the mouth area along the sympathetic nerve shown on the dog with permanent salivary fistulas was verified on acute experiments and fully confirmed. But, of course, it was also necessary to create the conditions requisite for its appearance. When in acute experiment with the dog, we cut the chorda tympani we did not obtain the reflex by rubbing the tongue and other parts of the mouth with wadding moistened in a 0.5 p.c. solution of HCl. It was, however, only necessary to inject pilocarpine or, instead of it, to excite by electric current the peripheral end of the cut chorda, to call forth the reflex action along the sympathetic nerve. I give below extracts of the acute experiments.

*Exp. 1.* Dog anaesthetised with chloroform and morphia. Duct of the left submaxillary gland prepared, a cannula is introduced into it, and this connected with a graduated glass tube. Pilocarpine injected. The movement of saliva after the chorda was cut, in every 30 seconds in the divisions of the tube, was as follows:

4½, 4, 3½, 4½¹, 8, 6, 4, 5, 4½, 5, 3, 3.

¹ Rubbing the mouth with a .5 p.c. solution of hydrochloric acid.

After the left cervical vago-sympathetic nerve had been cut, the saliva flow was:

3, 4, 3½, 3½, 3, 4¹, 3½, 2½, 3, 2½, 3½.

¹ Rubbing the mouth with a .5 p.c. solution of hydrochloric acid.

*Exp. 2.* Dog was first anaesthetised with æther and chloroform—then curari and morphia were used. The method was the same as in Exp. 1 but the peripheral end of the chorda tympani was stimulated by faradic currents for 2½ minutes instead of injecting pilocarpine. When the after flow of saliva was slow acid was placed in the mouth.

17. 2½, 3, 13½¹, 6, 1, ½, ½.

¹ Rubbing the mouth with a .5 p.c. solution of hydrochloric acid.

After this the cervical vago-sympathetic was cut and the stimulation of the chorda was continued for four minutes.

The after action: 3,  $1\frac{1}{2}$ ,  $1\frac{1}{2}$ , 1<sup>1</sup>, 0,  $\frac{1}{2}$ , 0<sup>1</sup>, 0.

<sup>1</sup> Rubbing the mouth with a solution of .5 p.c. of hydrochloric acid.

The last experiment is specially valuable because no secretory poison was given and the stimulus was strictly localised in the area of chorda tympani.

In view of the above-mentioned facts, we must conclude that a certain degree of preliminary irritability of the gland is necessary for the manifestation of the capacity of the sympathetic nerve to transmit the reflexes from the mouth region. In the normal working of the gland this condition exists when the gland is stimulated by the cranial nerve; the sympathetic can then join in the secretory action. Although the nervous impulses passing by the sympathetic in the mouth reflex have no visible effect in the absence of a certain degree of gland excitability, it does not follow they have no effect. It is possible that with more delicate methods or by microscopic examination some changes may be observable.

We may now consider the "augmented secretion" obtained by the irritation of the sympathetic nerve demonstrated in 1888-1889 by Langley(10) (see also Bradford(9)). If we give our attention to the extract of my Exp. 2 in which the reflex through the sympathetic nerve arose after preliminary excitation of the chorda tympani—we find a remarkable resemblance between my row of figures and the tables shown by Langley(10) for the illustration of "augmented secretion<sup>1</sup>." The only apparent difference is that in my experiments the more natural method of excitation was employed.

In the text books of physiology, the chapter on "augmented secretion" is isolated, and the "augmented secretion" is not mentioned in drawing up the general conclusions. It is now clear that the "augmented secretion" is only a special manifestation of the capacity of the sympathetic nerve and that it has a deep functional meaning.

In reflex movements of the body, different stimuli work together to a common end; the resulting movement is a conjoint effect and not the result of simple summation of stimuli—a point which has been developed by Wedensky(11, 12). In the salivary glands there is a similar conjoint action, but one which involves two nervous systems, the para-sympathetic and the sympathetic. So far I have only shown that the conjoint action

<sup>1</sup> In this work (p. 325) Langley has shown, apart from this, that during the pilocarpine salivary secretion of dog's parotid gland, the stimulation of the sympathetic nerve is exhibited in a precise secretory effect.



affects the quantity of the saliva, but it is probable that it occurs also in the production of the organic substance. This I hope to investigate later.

Some experiments of Malloizel<sup>(13)</sup> bear on the questions discussed here. Malloizel made one observation on the effect of feeding in a dog with a fistula of the mixed glands after section of the chorda tympani and found that it caused no secretion. On the other hand he found that feeding still caused a slight secretion—3 to 4 drops—after atropine had been given. This effect he did not obtain in another dog after excision of the superior cervical ganglion. These results suggest a reflex secretion by way of the sympathetic. Malloizel's atropine experiments were repeated by Babkin<sup>(6)</sup>. Babkin found that atropine in the late stages of its action prevented any secretion being obtained by feeding. In the early stage, 1-2 drops of saliva were obtained. This might have been due to imperfect paralysis of the chorda tympani or more probably, in view of my experiments, to the excitability of the gland being sufficient to allow an augmented secretion to be produced by way of the sympathetic.

#### SUMMARY.

1. The sympathetic nerve is capable of transmitting reflexes from the mouth area, and has an important significance in the process of normal saliva secretion.

2. The action of the sympathetic nerve manifests itself in conjoint action with the cranial nerve. Thus nervous impulses reaching the glands through the sympathetic and the parasympathetic paths can act not only antagonistically, but also can give each other mutual support.

3. The nature of the stimulus in the mouth region influences the amount of the activity of the secretory function of the sympathetic.

In conclusion, I express my thanks to Professor B. P. Babkin for the suggestion of this work and his advice throughout.

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## CREATINE FORMATION DURING TONIC MUSCLE CONTRACTION. BY K. UYENO AND T. MITSUDA.

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### *Creatine of the amphibian muscles in the breeding season.*

KAHN(1) found less creatine in the flexor muscles of the arm and a part of the pectoral muscle than in the muscles of the leg in the male frog while clasping the female in the breeding season. He interpreted this fact as a decrease of creatine in muscles contracting tonically. Riesser(2) showed that this interpretation was not justified since in the frog the normal content of creatine is less in the arm than in the leg muscles, but he considered that creatine was not produced by the clasping contraction. The contraction was held by Kahn to be tonic on account of the absence of action currents.

As our experiments with nicotine(3) suggested that creatine is formed during the tonic contraction of frog's muscle it seemed desirable to investigate the question on the clasping muscles. The fact shown by Riesser is inconclusive as he did not compare the creatine of the normal frogs with that of the clasping frogs in the same season. Our scheme was, therefore, to compare the creatine of the arm and leg muscles in the breeding season in frogs and toads which were clasping and those which were not. Muscles taken for analysis were the flexor muscles of the arm and the pectoral muscles on one hand and the whole muscle of the hind limb, except small muscles of the foot, on the other. The muscles of the two sides were taken. Creatine was estimated as creatinine by Folin's method. For the comparison we use the quotient

$$\frac{\text{creatine in clasping muscles}}{\text{creatine in hind limb muscles}} \times 100,$$

since the creatine content of the normal frogs varies in fairly wide range so that it may sometimes cover the range of actual increase by tonic contraction<sup>1</sup>.

Experiments were begun with toads. Males during clasping, most freshly caught, some from the laboratory stock, were observed for several

<sup>1</sup> In accordance with custom we use the term "tonic contraction" for the persistent contractions dealt with in this paper, but we do so without prejudice as to the nature or frequency of the nerve impulses causing them.

muscles were cut and after 4 hours, during which the cats were anaesthetised with urcthane, the muscles were taken for analysis. In the last series the cat was decerebrated, the motor nerves to muscles being cut on one side before decerebration, and killed after four hours. In all cases creatine was estimated as creatinine by Folin's method.

The results of the first series are shown in Table III. From this we see that the difference of the two sides is very slight though the absolute values differ in different muscles as well as in different cats. The percentage difference varies from 0 to 3.5. Roughly speaking there is a difference of about 2 p.c. Hence if we find any difference greater than this we may probably attribute it to an altered condition of the muscles.

In Table IV we show the results of the second series. The amount of creatine was always less on the side on which the motor nerves were cut. Except in two cases of the triceps muscle, in one of which we noticed a distinct stiffness of the muscle on the side with intact motor nerve, the percentage difference was about 3. The decrease of creatine in paralysed

inine (in p.c.) in decerebrate rigidity (cat).

TABLE III. Comparison of the two sides in normal cats.

	Cat	Right	Diff. left	P.c. diff.
brachii	1	.385	-.007	1.8
	3	.513	-.007	1.4
	5	.450	+.004	.9
	6	.445	+.010	2.3
arcus	1	.396	-.005	
	2	.465	-.006	
	5	.421	-.006	
	6	.482	+.006	
nemius	2	.435		
	3	.533	-	
	4	.494	-	
	5	.495	-	
membranosus	4	.414	C	

TABLE IV. Section of motor nerves cats killed after 4 hours.

	Cat	Nerve intact	nerve
brachii	1	.656	-.006
	2*	.440	-.006
	3	.426	-.030
arcus	1	.615	-.018
	2	.421	-.009
	3	.440	-.014

the fore leg was stiff and kept thrust out. Unknown.

TABLE V a. Decerebration, rigidity developed strongly, kept 4 hours.

	Cat	Non-rigid	Diff. rigid	P.c.
Triceps brachii		.519	+.052	10
		.481	+.040	8
		.455	+.040	8
Vastocurcureus			+.045	7
			+.035	7
			+.059	13
Gastrocnemius			+.033	
			+.020	
Soleus			+.015	
			+.015	
* Rigidity in the fore leg in the hind				

TABLE V

	slight rigidity (C)
Triceps	-.006
	+
Gastrocnemius	+

muscle may be, as suggested by various workers, referred either to the increased blood flow and consequent increased washing away of creatine or to a decreased formation of creatine in the paralysed muscles, or to both of them. This question we have not investigated.

The results of the third series of the experiments are shown in Table V a. The percentage increase of creatine in the rigid muscles was 7.7 to 13.2 in the triceps and vastocrureus and much less (about half) in the gastrocnemius and soleus. Pekelbaring and Hoogenbuyze—who abolished rigidity by cutting posterior roots—found 7.9 to 19.4 p.c. in the triceps and Dusser de Barenne and Tervaert,—also cutting posterior roots, 1.5 to 9.7 in triceps and 1.9 to 1.1 in gastrocnemius. We may point out that the difference of creatine was much less in the gastrocnemius, that this was corresponding to the less degree of rigidity, and that it was on the gastrocnemius that most of the experiments by Dusser de Barenne and Tervaert were made. The cases of decerebration in which rigidity was slight and not persistent or did not develop at all are shown in Table V b. In these cases there was no distinct increase of creatine. Our results we think show that decerebrate rigidity is accompanied by a definite increase in creatine formation.

#### SUMMARY.

1. In male toads and frogs creatine was found to increase in the claspings muscles during coupling in the breeding season.
2. In decerebrate rigidity creatine increases in the rigid muscles.

We wish to thank Prof. Langley and Prof. Hopkins for the facilities given us in their laboratories.

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without food, though given water, for a period of 18-20 hours. At the end of this time, on the first occasion, a subcutaneous injection of

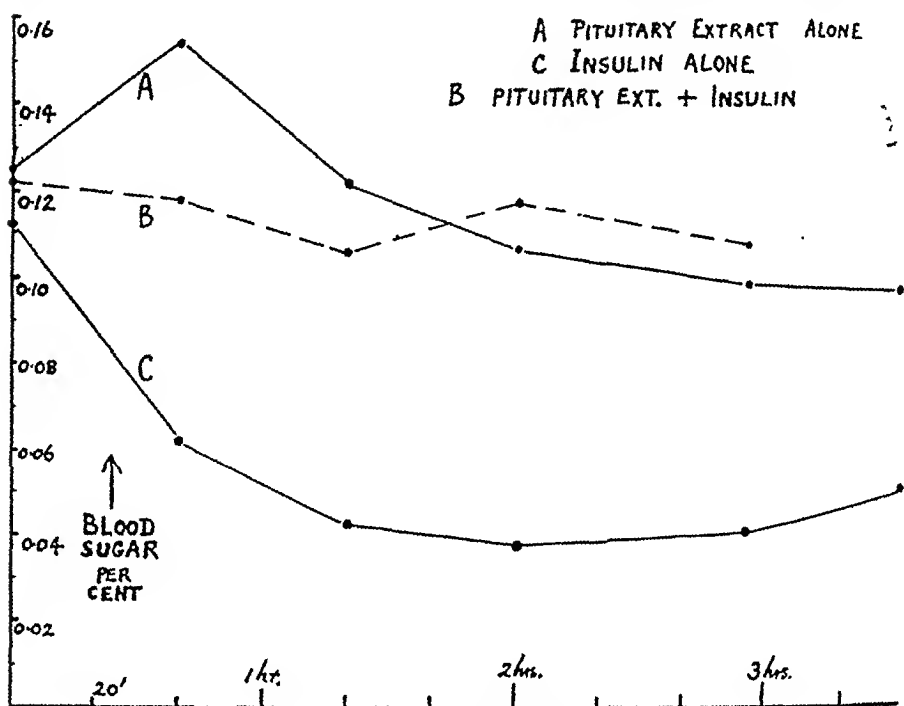


Fig. 1. For description see text.

4.7 mgm. of a batch of insulin was given. Samples of blood were withdrawn from the ear vein before the injection, and at various intervals after, and these showed that, in 2 hours from the injection, the percentage of blood sugar had fallen from .112 to .038. On the second occasion the same dose of insulin was injected into the loose skin of one flank, and immediately afterwards 4 c.c. of a 2.5 p.c. extract of fresh infundibular material were similarly injected into the opposite flank. At the end of 2 hours the percentage of sugar in the blood had not fallen at all, but, within the error of the method, was at the same level as before the injections took place. On the third occasion a similar dose of infundibular extract to that previously used was injected by itself, in order to demonstrate its effect on the blood sugar. After a relatively small initial rise, obvious at the end of 40 minutes, the blood sugar actually fell, being at the original level in 80 minutes, and clearly below it in 2 hours; the fall was maintained during the third and fourth hours.

The results obtained with rabbit 9, using a different preparation of

insulin and a commercial sample of posterior lobe extract, were in every way similar to those described for rabbit 7. Such results were confirmed on several other rabbits and demonstrated that injections of infundibular extract were able, according to the dose given, either to diminish or to abolish the fall produced by an injection of insulin.

The cases shown in Table I made it additionally clear that this effect of infundibular extract was not to be explained by its power when given alone of producing an increase of the blood sugar which, by algebraic summation, could compensate for the fall produced by insulin.

With reference to the effect of pituitary extract on the normal blood sugar, the behaviour of rabbit 7 was of some interest. The effect of subcutaneous injection, if any, is usually stated to be a rise. In the three experiments of this kind on rabbit 7, there was, in each case, a transient rise of blood sugar, followed in 2 hours by a definite though slight fall, lasting at least 2 hours longer. In two other rabbits, again, the *only* effect of subcutaneous injection of pituitary extract was a slight fall. In five other experiments, there occurred a simple rise of blood sugar of brief duration, and finally, in two experiments, the rise was considerable and prolonged. While, therefore, these experiments confirmed the view that in some cases pituitary extract injected subcutaneously does cause hyperglycæmia, they also showed that, in others, the opposite condition may result, and that, even in these, the simultaneous injection of pituitary extract prevents the fall of blood sugar caused by insulin.

*The specific nature of the effect.* Extracts of spleen, thymus, brain tissue, desiccated thyroid material, and anterior lobe of the pituitary gland were prepared and tested. With the exception of those from the thyroid and the anterior lobe, the extracts were obtained from the fresh tissues of the rabbit; the extract of thyroid was made from desiccated sheep's thyroid, and the anterior lobe extract from fresh ox pituitaries. The extraction was in all cases identical with that adopted for infundibular material, namely an extraction of weighed minced tissue, with a known amount of water, which was then faintly acidulated, boiled and filtered. The results with these extracts were uniformly negative; none of them affected, in either direction, the action of insulin, as determined in a control experiment on the same animal with the same dose of the same batch of insulin.

Since it is known that histamine occurs in many organ extracts, an experiment in which 3 mgm. histamine di-phosphate was injected simultaneously with a small dose of insulin was carried out. Again the

result in no way differed from that of the control experiment without histamine.

A final proof of the specificity of the action of infundibular extract was obtained by taking advantage of the fact (Guggenheim(8), Dudley(9)) that the oxytoxic and pressor principles present in this extract are destroyed by exposure for 2 hours to the action of normal sodium hydroxide at room temperature. A sample of pituitary extract treated in this way and subsequently neutralised, was found to have lost not only its activity on the isolated guinea pig's uterus, but also the power to prevent the fall of blood sugar produced by insulin. The following experiment illustrates this:

To a volume of posterior lobe extract, an equal volume of 2N NaOH was added. The mixture was allowed to stand for  $1\frac{1}{2}$  hours at 37° C. and then neutralised. A volume of this solution, representing a dose of the original extract known to abolish the fall produced by a given dose of insulin, was then used for injection. The control experiments are shown:

Rabbit 10. Wt. 3.7 kgm.

	Injections		
	7.4 mgm. Insulin A + 3 c.c. 1 % ext. of dried infundibular material	7.4 mgm. Insulin A + same dose of inactivated infundibular extract	
Blood sugar %	7.4 mgm. Insulin A alone		
Before injection	.123	.169	.138
40 mins. after	.085	.196	.084
60     "	.080	.188	.082
80     "	.086	.174	.079
100    "	.087	.171	.085
160    "	—	.139	.084

The third column of figures shows that after treatment of the pituitary extract with soda, its effect on the fall of blood sugar due to the insulin had disappeared. Hence it was evident that the ability of pituitary extract to suppress the effect of insulin depended on its content of a substance having the same instability to alkali as the known active principles.

*The effect of pituitary extract on hypoglycæmia.* As Banting, Macleod and their colleagues(10) have shown, a rabbit, after a large dose of insulin, passes into a condition in which periods of collapse alternate with periods of violent convulsions. It was found that, provided the dose was adequate, subcutaneous injection of infundibular extract never failed to remove all these symptoms in not more than 10 minutes from the time of injection, the recovery closely resembling that observed after the injection of dextrose. The following experiments, in which relatively

large doses of posterior lobe extract were used, show that the disappearance of convulsions was in fact attended by a rapid elevation of the previously low percentage of sugar in the blood

1 Rabbit. Wt 2.6 kgm (see Fig 2)

Injection	Time	Symptoms	Blood sugar
20 mgm insulin	Zero	—	134
	1 hour	—	051
	1½ hours	Temporary convulsions	—
	2 "	—	048
	From 2 h 40 m to 2 h 57 m	Continuous convulsions and collapse	—
5 c c 10 % commercial infundibular extract	2 h 57 m.	—	—
	3 h 7 m	Normal	—
	3 h 20 m	—	092

2 Rabbit. Wt 2.6 kgm

Before injection of insulin	Blood sugar	109 p c
After injection 1 hour	"	047
" 2 hours	"	045
" 3 "	"	035
" 3 h 5 m	Convulsions	
" 3 15	4 c c 2 p c extract dried infundibular material injected subcutaneously	
" 3 20	Convulsions	
" 3 25	Rabbit sits up and appears normal	
" 3 35	Blood sugar	065 p c

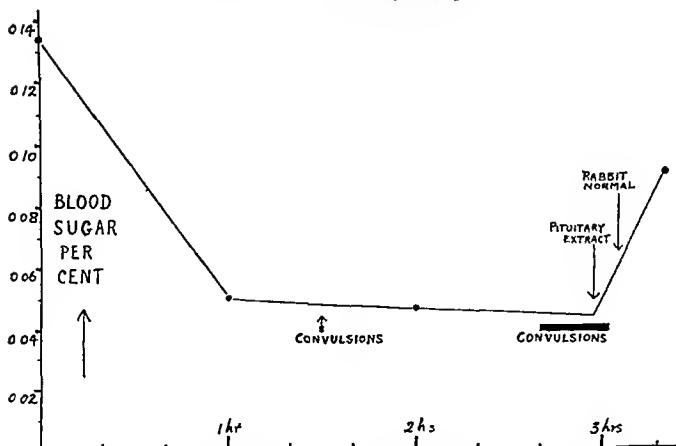


Fig 2 Effect of pituitary extract on the blood sugar of an animal in hypoglycæmic convulsions Insulin injected at zero



In the first of these experiments a rabbit received an injection of insulin great enough to produce, after 160 minutes, a series of convulsive fits, in the intervals between which the animal was seen to be in a condition of collapse. At this point the percentage of blood sugar was certainly not greater than .048 p.c., a value observed one hour previously, and probably was below .04 p.c. Subcutaneous injection of pituitary extract led to the complete recovery of the animal within 10 minutes, and a sample of blood withdrawn 23 minutes after the injection contained .092 p.c. sugar.

*The effect of adrenaline on insulin hypoglycæmia.* Since it has already been shown by Banting, Best, Collip, Macleod and Noble(11) that there exists an antagonism between the effect on the blood sugar of adrenaline and that of insulin, some experiments were carried out to see what points of difference there were between the action of adrenaline and of pituitary extract in this respect. The observations on the relation of adrenaline and insulin fully confirmed what was found by the Toronto workers, for it was clear that the hyperglycaemic effect of a given dose of adrenaline was greatly reduced when the dose was given to an animal already under the influence of insulin. The experiments

TABLE II. Rabbit 1. Wt. 3 kgm. On occasion of each experiment, it was kept without food for 18-20 hours previously.

Exp.	Time	Blood sugar %	Injection (subcut.)	Exp.	Time	Blood sugar %	Injection (subcut.)
A	Just before injection	.093	—	D	Just before injection	.123	—
	Zero	—	12 mgm. insulin B		Zero	—	12 mgm. insulin B
	100 m. later	.044	—		158 m. later	.050	—
	170 "	.034	—		166 "	—	0.5 mgm. adrenaline
	220 "	.031	—		186 "	.051	—
	280 "	.052	—		211 "	.069	—
					255 "	.077	—
B	Just before injection	.110	—				
	Zero	—	.5 mgm. adrenaline	E	Before injection	.121	—
	38 m. later	.169	—		Zero	—	12 mgm. insulin
	63 "	.233	—		40 m. later	.064	—
	90 "	.291	—		80 "	.053	—
	128 "	.337	—		100 "	.041	—
					158 "	.036	—
C	Before injection	.120	—		160 "	—	4 c.c. "Infundin"
	Zero	—	4 c.c. "Infundin"				
	20 m. later	.152	—		182 "	.084	—
	50 "	.157	—		210 "	.071	—
	90 "	.113	—		254 "	.066	—
	120 "	.124	—				

described in Table II were all carried out on one rabbit. They show the effects of insulin, pituitary extract and adrenaline when given singly, as well as the antagonism of the two last for insulin. It will be seen that the effect of the chosen dose of adrenaline alone in producing hyperglycaemia is not only much greater ( $\cdot 110$ – $\cdot 337$  p.e.) than that of the dose of pituitary extract ( $\cdot 120$ – $\cdot 157$ ) but much more prolonged; on the other hand the antagonistic effect of the same dose of pituitary extract on insulin hypoglycaemia is more extensive, more persistent and much more rapid in onset than that of adrenaline.

*The effect of pituitary extract on adrenaline hyperglycaemia.* In view of these effects, it seems relevant to give an example (Table III) showing the relation between infundibular extract and adrenaline on the blood sugar of a normal animal. As already stated, Stenström<sup>(4)</sup> originally described the inhibition of adrenaline hyperglycaemia and the suppression of adrenaline glycosuria in rabbits by infundibular extract. These findings were confirmed by me in experiments, of which the details were not published.

TABLE III. Rabbit. 2.8 kgm. In each case food was withheld for 18–20 hours before the experiment.

Time	Blood sugar percentages	
	After injection of .5 mgm. adrenaline	After injection of 5 mgm. adrenaline + 4 c.c. pit. ext. "Infundin"
Before injection	$\cdot 107$	$\cdot 111$
40 m. after	$\cdot 195$	$\cdot 127$
80 "	$\cdot 277$	$\cdot 123$
120 "	$\cdot 329$	$\cdot 144$
180 "	$\cdot 325$	$\cdot 198$
240 "	$\cdot 269$	$\cdot 252$

The changes observed are characteristic of those seen in these circumstances; some evidence of the adrenaline action is usually visible in spite of the injection of infundibular extract. Nevertheless at the end of 2 hours from the injection, Table III shows that a rise due to adrenaline alone amounting to  $\cdot 22$  p.c. has been reduced, under this inhibiting influence to one of only  $\cdot 03$  p.c. A similar effect is observed on the hyperglycaemia produced in rabbits by ether anaesthesia provided that the dose of pituitary extract is large, and is administered before the induction of the anaesthetic.

*The effect of ergotoxine on adrenaline hyperglycaemia and on insulin hypoglycaemia.* There are two additional experimental results which have significance for an analysis of the relation of the factors which influence

the circulating glucose. The first of these was originally described by Miculicich(12) and confirmed by myself; it was that the glycosuria and hyperglycæmia produced by adrenaline are inhibited by an intravenous injection of ergotoxine. The suppression is more nearly complete than that produced by pituitary extract. It was accordingly of interest to examine the effect of ergotoxine on insulin hypoglycæmia. Instead of ergotoxine, ergotamine tartrate was used, which has recently been shown by Dale and Spiro(13) to be identical, quantitatively and qualitatively, in its physiological properties with ergotoxine. It was found that following an intravenous injection of ergotamine, the amount of insulin adequate to produce convulsions was much less than in the normal animal. Fig. 3 shows the course of the changes in the blood sugar in two experiments on a well fed rabbit. In one experiment (A) 8 mg. of insulin was given alone. In the other (B) 5 mgm. of ergotamine tartrate was first given and 2 hours later 8 mgm. of the same sample of insulin.

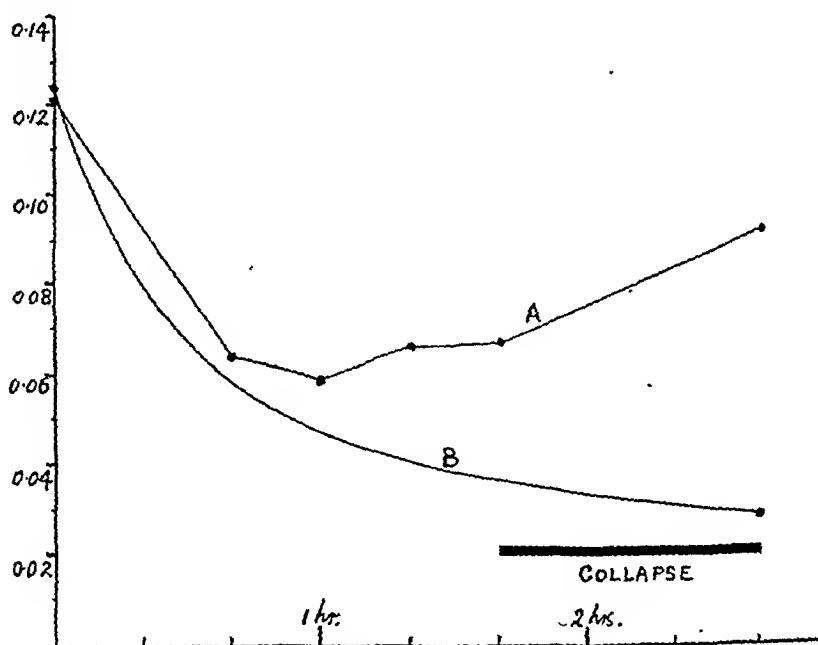


Fig. 3. Curve A shows the effect of a small dose of insulin on the blood sugar of a well-fed rabbit. Curve B shows the effect of the same dose given 2 hours after an intravenous injection of 5.0 mgm. ergotamine tartrate in the same animal.

It will be seen that the injection of insulin alone produced a transitory fall, 1 hour later, to the neighbourhood of .060 p.c., and the last traces of its effect were disappearing in 2 hours 40 minutes, and that following

the ergotamine administration, the sugar, under the influence of the same amount of insulin, fell to  $\cdot 029$  p.c.; the animal passed from convulsions to a condition of complete collapse, so that blood was only obtained for analysis by cardiac puncture; the animal would certainly have died but for an intravenous injection of dextrose.

### *Discussion.*

While it is a matter of some difficulty to fit all of the preceding results into a consistent scheme, there is at least one action the mechanism of which seems reasonably certain. It is generally agreed that the hyperglycæmia produced by adrenaline is due to conversion of liver glycogen into glucose, and the fact that this, like other "motor" effects of adrenaline, is paralysed by ergotoxine, shows that it is due to action on a mechanism in the liver associated with sympathetic innervation; in other words, the adrenaline hyperglycæmia is simply one of its sympathomimetic actions.

The recent work of Macleod and his colleagues<sup>(14)</sup> suggests that while the initial fall of blood sugar caused by insulin is independent of the amount of liver glycogen, the rate at which the blood sugar returns to its normal level, as the effect of the insulin passes off, is largely determined by the amount of sugar which the liver can supply. This view is fully confirmed by the observation that ergotoxine greatly increases the action of insulin; it may be supposed that the alkaloid, by paralysing the sympathetic supply to the liver, prevents this organ from carrying out its compensatory function, so that a small dose of insulin has not to deal with a continuous supply of sugar from the liver, and is therefore able to produce an almost complete disappearance of circulating glucose.

This view further explains the observations here recorded with adrenaline. The experiments described show that the hyperglycæmic action of a given dose of adrenaline in the normal animal is reduced to one-sixth of its value in the same animal 2 hours after the injection of insulin. If in this condition the liver is already producing sugar as rapidly as possible, the additional stimulus of adrenaline will be proportionately less effective.

There remains the relation of pituitary extract to carbohydrate metabolism. Cushing's work suggests that the specific antagonism, between the active principle of the pituitary posterior lobe and insulin, here described, is a factor of importance in normal metabolism. Cushing found that the patient suffering from hypopituitarism, or the animal

deprived of its pituitary body, developed a condition which was the antithesis of that of the diabetic. There was an enormously increased sugar tolerance which led to adiposity, and frequently there was hypoglycæmia. We have now evidence that pituitary injections inhibit the action of insulin in producing hypoglycæmia, and that, when given to an animal in hypoglycæmic convulsions, they produce a rapid recovery, due to return of the blood sugar towards the normal. These facts suggest that carbohydrate metabolism is influenced by pituitary extract in a precisely opposite sense to that in which it is affected by insulin.

It is not yet clear to what the hypoglycæmia produced by insulin is due. On the one hand there is the evidence of Dixon and Pember, quoted by Macleod and others<sup>(14)</sup> of a raised respiratory quotient following the injection of insulin, and on the other hand the evidence of Dudley, Laidlaw, Trevan and Boock<sup>(15)</sup>, who have described a diminished respiratory exchange, suggesting that the sugar is dealt with in some other way than by combustion. If insulin were to promote increased combustion of sugar, we should conclude that pituitary extract prevented this taking place; but then, and indeed on any available view of the mechanism of the action of insulin, it is difficult to explain the origin of the sugar which is almost immediately liberated in the system of a hypoglycæmic rabbit by an injection of pituitary extract. It is extremely hard to believe that the source of this is the liver. We know that pituitary extract prevents, in rabbits, not only the glycosuria and hyperglycæmia due to adrenaline, but also that due to anæsthetics, in both of which cases pituitary extract must have a deterrent effect on glycogenolysis. It does not appear credible that pituitary extract should prevent adrenaline hyperglycæmia by suppressing the conversion of glycogen into sugar, and also prevent insulin hypoglycæmia by accelerating the same process<sup>1</sup>. Further discussion of the exact meaning of the antagonism between pituitary extract and insulin must obviously be postponed until the mode of action of insulin itself is more completely understood. The point here to be emphasised is that while adrenaline apparently opposes the action of insulin simply in virtue of its accelerating effect on the normal compensatory action of the liver, the antagonism of pituitary extract for insulin is of a more direct and specific nature, and is not shared by extracts of the other tissues which have been examined.

<sup>1</sup> Direct evidence against the view that insulin hypoglycæmia is due to glycogen formation is shortly to be published by Dr H. W. Dudley.

## SUMMARY.

1 Subcutaneous injections of extract of posterior lobe of the pituitary gland given simultaneously with injections of insulin, diminish or abolish the fall of blood sugar produced by the latter. The doses of pituitary extract used do not, when given alone, produce a rise of blood sugar sufficient to explain this inhibition of the action of insulin as being the result of an algebraic sum.

2 This effect is not produced by similar extracts of anterior lobe of pituitary, or of spleen, thyroid, brain tissue or thymus. Nor is it produced by histamine. The property, in the case of posterior lobe extract, is destroyed by treatment with *N*-alkali in the cold.

3 Pituitary extract removes the symptoms of hypoglycæmic convulsions, causing a rapid elevation of the blood sugar.

4. The effect of a small dose of insulin is greatly increased by previous intravenous injection of ergotoxine.

5 Confirmation is given of the following points: (a) pituitary extract inhibits adrenaline hyperglycæmia and glycosuria, (b) ergotoxine abolishes adrenaline hyperglycæmia and glycosuria, (c) insulin greatly reduces the hyperglycæmic action of adrenaline.

6 Cases are given in which subcutaneous injections of pituitary extract in the normal animal led to a fall of blood sugar, though as a rule such injections cause a transient rise.

My thanks are due to Dr H. W. Dudley for the samples of insulin used, and to Mr H. P. Marks for carrying out some of the estimations.

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## THE EFFECT OF TEMPERATURE ON THE ISOLATED IRIS OF THE CAT. BY CATHARINE A. VERBITZKY.

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THE effect of changes of temperature on the iris does not seem to have been fully studied either by older (Brown-Séguard<sup>(1)</sup>, Muller<sup>(2)</sup>, Schur<sup>(3)</sup>, Samkow<sup>(4)</sup>, Grünhagen and Samkow<sup>(5)</sup>) or by recent (Ten-Cate<sup>(6)</sup>) investigators, and it therefore seemed desirable to follow the effect of temperature over as wide a range as possible.

*Method.* The isolated iris of the cat, removed from the eye enucleated under anaesthesia, or immediately after death, was employed; the iris was excised from the enucleated eye as soon as possible after its removal, and was transferred to a glass vessel filled with Tyrode's solution, one edge of the iris being pinned to a cork fitted to the bottom of the vessel, while to the pupil was attached a small platinum hook, connected by a thread to a straw lever 30 cm. in length and 0.3 g. in weight. The movements of the lever were recorded on a blackened drum. In order to load the iris, riders of different weights were made, and by moving these along the long arm of the lever, which was divided in centimetres, the loading could be widely varied. The most suitable load was found to be .08 g. The heating of the vessel was carried out by means of a small spirit lamp placed beneath it, and the temperature of the saline solution recorded on the tracing every minute. A time tracing was also made. Experiments, of which in all 43 were made, were carried out both in winter and in summer. In summer, ice was used for cooling, while in winter, cooling proceeded spontaneously in the unheated laboratory, the temperature of which was from 2° to 0° C. In most of the experiments aeration of the saline solution was not performed, because control experiments showed that bubbling of air through the solution made no essential difference to the results, except that in the latter case the observed relaxation occurred earlier. In all the figures one division of the ordinate corresponds to 0.5 mm. of the tracing, one division of the abscissa to 30 seconds.

*Effect of heating.* Fig. 1 gives an analysis of a characteristic curve; the height of the tracing was noted each minute with an accuracy of

0.5 mm. The temperatures are written on the curve, the initial temperature being  $11^{\circ}\text{C}$ . Contraction of the iris began at  $19^{\circ}$ , and reached

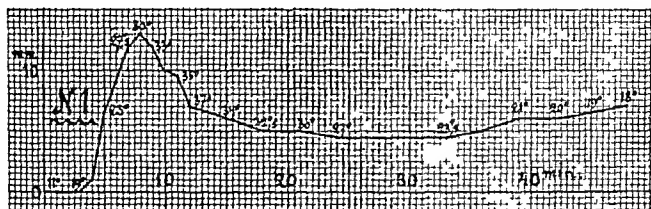


Fig 1

its maximum at  $30^{\circ}$ , after which it fell, in spite of the heating being continued to about the normal body temperature. Subsequent cooling led to a slow increase of tone. Unmistakable spontaneous contractions of the tissue, such as have been described by Ten-Cate (6, 7), were never seen. Whether this is owing to lack of oxygen, or to too heavy a lever, or to the fact that the cat's iris is not a suitable object for their demonstration, I am unable to say (see Fecstra (8))

In Fig. 2 is shown the effect of warming the excised iris from  $2^{\circ}$  to

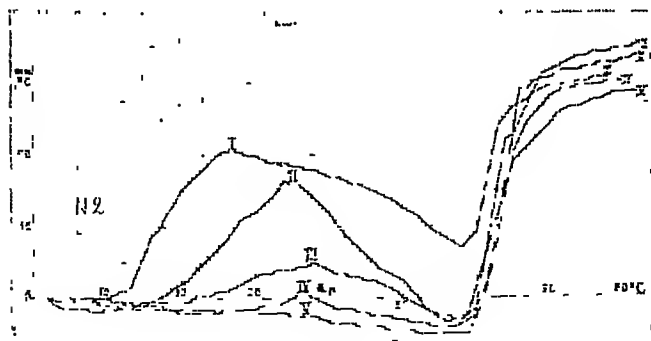
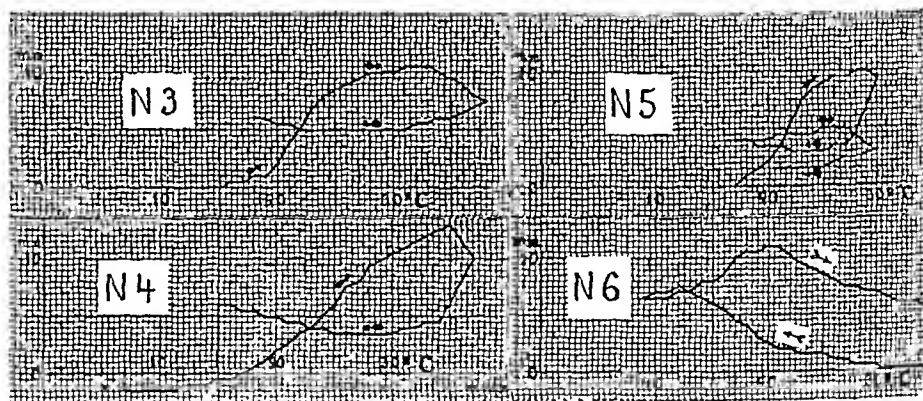


Fig 2

$80^{\circ}\text{C}$ . with levers loaded to different extents. Curve I load = 0.06 g., curve II = 0.08 g.; curve III = 0.08 to 0.15 g.; curve IV = 0.2 g.; curve V



= .35 - .4 g. These curves represent the averages of a number of experiments; the time factor was omitted. At the lower temperature the muscle of the iris is in a relaxed state, and regained tone on warming, the maximum tone being at about  $30^{\circ}$  ( $27^{\circ}$ - $35^{\circ}$ ). Further rise of temperature led to a fresh relaxation which reached its limit at about  $60^{\circ}$ . Above this temperature there occurred a sudden contraction from 1.5 to 2 times as great as that at  $30^{\circ}$ ; this sudden rise was due to the onset of heat rigor, and was followed by a further slow contraction. The type of these curves is not affected by alteration of the load, unless this be excessive; but the actual degree of shortening at  $30^{\circ}$  is seen to be reduced when the load is increased. It is also seen that the greater the load, the more pronounced the relaxation at about  $60^{\circ}$ , so that the onset of heat rigor appears to be more sudden. But the onset of heat rigor appears at the same temperature whatever the load.



Figs. 3-6.

*The effect of cooling after previous heating, and vice-versa.* When the iris is cooled after being heated, there is but little alteration in length when the load on the lever is a light one (Fig. 3), but with more heavily-loaded levers, there is pronounced relaxation (Fig. 4). On once more warming the preparation there is further shortening, though much less distinctly than the first, and reaching its maximum earlier (Fig. 5); if this is again cooled, the relaxation is still more marked, and is uninterrupted by any rise at all. In each case the curves represent the average of a number of experiments.

In carrying out the reverse experiment, the iris was at once placed in Tyrode's fluid at  $35^{\circ}$ ; its tonus was then but weak, but was augmented by cooling until  $12^{\circ}$  was reached, below which it again began to fall off.

On re-warming the tonus was increased until  $20^{\circ}$ , after which it again showed relaxation (Fig. 6).

*The part played by the connective tissue of the iris in the contractions.* It seemed desirable to ascertain to what extent the contractions induced in the tissue of the iris by warming to  $30^{\circ}$  C. or above  $60^{\circ}$  C. respectively were due to change of tone in the muscle, and how far they could be explained as due to alterations in the connective tissue incorporated in the preparation employed. The hulk of the muscle is confined to the inner portion of somewhat less than half of the whole iris, the iris was therefore cut into two concentric rings, the inner and smaller of which contained chiefly muscle, while the outer and larger one consisted chiefly of connective tissue.

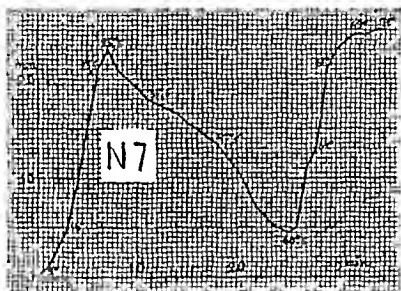


Fig. 7.

Fig. 7 shows the result of heating the inner ring, and it is seen that this behaves essentially in the same way as the whole iris, showing a contraction at  $25^{\circ}$  C. and another one due to heat rigor, and scarcely larger than the first at  $60^{\circ}$  C. The outer portion of the iris, the effect of heat on which is illustrated in Fig. 8, is seen to behave quite differently; there is no contraction in the middle range of temperature, while the effect of heat rigor is very definitely expressed. We may conclude therefore, that the first contraction shown by the excised iris on heating is wholly due to an increase in the tone of its muscular tissue, while the heat rigor shortening is very largely due to coagulative changes in the connective tissue of the outer part of the structure. This accords with the observation made long ago by Mourasheff(9) and more recently confirmed by Bottazzi(10) that connective tissue shows considerable shortening above  $60^{\circ}$ .

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AN ANALYSIS OF THE EFFECTS OF SPEED ON THE  
MECHANICAL EFFICIENCY OF HUMAN MUSCULAR  
MOVEMENT. BY HARTLEY LUPTON<sup>1</sup>.

WITH AN APPENDIX BY A. V. HILL.

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THE factors underlying the effects of speed on the mechanical efficiency of human muscular movement have been discussed by A. V. Hill (1, 2). The efficiency  $E$  is defined as  $W/H$ , where  $W$  is the actual work done, and  $H$  the total energy liberated by the body during, and in recovery from, the effort involved. In a particular movement—the flexion of the arm in the supinated position—it was shown experimentally by Hill, and confirmed by Lupton (3), that the work done increases as the speed of movement decreases, according to the formula  $W = W_0 \{1 - (k/t)\}$ ;  $W_0$  and  $k$  are constants, and  $t$  the time occupied in the movement. The quantities  $W_0$  and  $k$  have been given a theoretical meaning by Hill, but apart from any theory the fact remains that the work done in a maximal movement is given, in terms of its duration, very accurately by the formula. At short durations the work done is zero or small; it increases rapidly at first as the duration is increased and then more slowly, attaining asymptotically the value  $W_0$  at long durations. The result was obtained in a maximal contraction, the only form of contraction in man in which it is possible (so it is assumed) to investigate a constant number of stimulated muscle fibres: there would seem, however, to be little doubt that, if a constant sub-maximal number of fibres could be stimulated the same type of result would be obtained. The quantity  $H$ , and its variation with the duration of the contraction, has been investigated on the muscles of the frog: Hartree and A. V. Hill (4) found that the formula  $Q = a + bt$  is accurately obeyed, where  $Q$  is the total heat liberated, per unit of force developed and maintained, in a contraction of duration  $t$ , and  $a$  and  $b$  are constants. The constant  $a$  is the energy required to “set up” the contraction,  $b$  is the energy required per second to “maintain” it. This formula again was established only for the case of a maximal contraction: there can be little doubt, however, that it

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would apply equally to a case in which a constant sub-maximal number of fibres was stimulated to a maximal degree. Hence, for the shortest possible contraction, a constant amount of energy is required (per unit of force developed) to set it up, and as the duration is increased the energy expended increases as a linear function of the duration. Hence in man we may write  $H = A + Bt$ . Now Hill has shown that, according to the formula,

$$E = \frac{W}{H} = \frac{W_0 \{1 - (k/t)\}}{A + Bt}$$

the efficiency varies with the duration of the movement, increasing (as the duration is increased) from a very low value in a very rapid movement to a certain maximum value, and then decreasing again as the movement becomes still slower. The existence of this optimum speed of movement, and the variation of efficiency with speed, are phenomena both of practical and of theoretical interest, and their closer analysis is desirable. The first part of this paper records an investigation in man in which the actual mechanical efficiency, directly determined from (a) the work done in raising the body, and (b) the oxygen used, is shown to vary with the duration of the movement in a manner conforming with Hill's expression. In the second part a quantitative experimental estimation is made of the constants  $W_0$ ,  $k$ ,  $A$ , and  $B$ , for one group of muscles, viz. the flexors of the elbow, and with these the maximum efficiency has been calculated and shown to agree well with that commonly observed in man.

## PART I.

The exercise chosen was the mounting of a given number of steps at various speeds. Without an almost unlimited flight of stairs, or a moving staircase, it is not possible to go on with this exercise, in a continuous manner, long enough for a "steady state" to be attained (Hill and Lupton (5), p. 148). It is necessary therefore to adopt the alternative method (*ibid.* p. 156) and to measure the total energy used during a given climb and in recovery therefrom. The mechanical efficiency then is  $E = W/H$ , where  $W$  is the work performed in raising the body through the height of the stairs, and  $H$  is the energy liberated calculated from the total excess oxygen used (over and above the resting level) from the beginning of the exercise to the end of the recovery period.

If  $W$  be kept constant, by raising the body always through the same height, then the efficiency is proportional to the reciprocal of the oxygen consumption produced by the exercise. It is in terms of this reciprocal that the efficiency is given in Fig. 1.

The procedure was as follows: after determining the standing respiratory exchange fasting, the subject stood for 3 minutes at the bottom of the stairs, with bag and valves in position, breathing to air. Immediately he began to ascend he turned the tap to the bag, the experimenter at the same time starting two stop-watches. One watch was stopped when the subject reached the top of the stairs; the other was stopped at the end of the recovery period, which extended in every case to at least 7 minutes after the end of the exercise. A longer recovery period was not required, as the exercise was mild and short lived and recovery therefore rapid. Recovery took place standing at the top of the stairs. The exercise was then repeated at a different rate, the subject first standing at the bottom of the stairs for 3 minutes before beginning the ascent. It was essential for the movement to be carried out smoothly and uniformly. Thus it was found that any extraneous movement whatsoever caused a reduction of the efficiency. The movements had to be made continuous and not jerky: that is to say, a very slow movement was required to be the exact replica of a more rapid one, simply with a different time-scale: it was essential that a slow step should not consist of a more rapid one followed by a pause. This condition was not always easy to satisfy: unless strict attention were paid to it the movement, at the slower speeds, was not spread out over the whole interval, the subject moving more rapidly and then standing freely on the next step. Six satisfactory experiments, however, were performed on different subjects, the following being typical.

TABLE I. Subject H. L., post-absorptive condition; height 168.5 cms.; weight (stripped) 58 kilos; vertical height of stairs 12.2 metres; number of vertical steps 78; weight with clothes, bag and valves 62 kilos.

Time taken in mounting stairs, secs.	23.6	24.3	29	34.5	41.8	49	69.5
Excess O <sub>2</sub> consumption resulting from exercise, c.c.	2796	2297	2030	1815	1529	1653	1510
Efficiency <sup>1</sup> proportional to	358	436	493	551	655	605	663
	85	99.5	154.5	172.5	213	225	237
	1462	1456	1504	1685	2234	2352	2387
	685	687	665	594	439	426	419
						419	403

<sup>1</sup> The efficiency is taken as proportional to the reciprocal of the oxygen consumption multiplied by 10<sup>6</sup>.

These results, given in Fig. 1, show that in the case of the muscle groups involved in the climbing of stairs there is an optimum speed at which the efficiency is a maximum. At the fastest speeds, *i.e.* when the time spent on the exercise is small, the efficiency is very low. At such speeds, on Hill's theory, a large proportion of the energy liberated is used in overcoming the internal resistance of the muscles themselves.

As the time spent in the exercise is increased the efficiency rises rapidly to a maximum. Then as the factor of maintenance of tension ("static

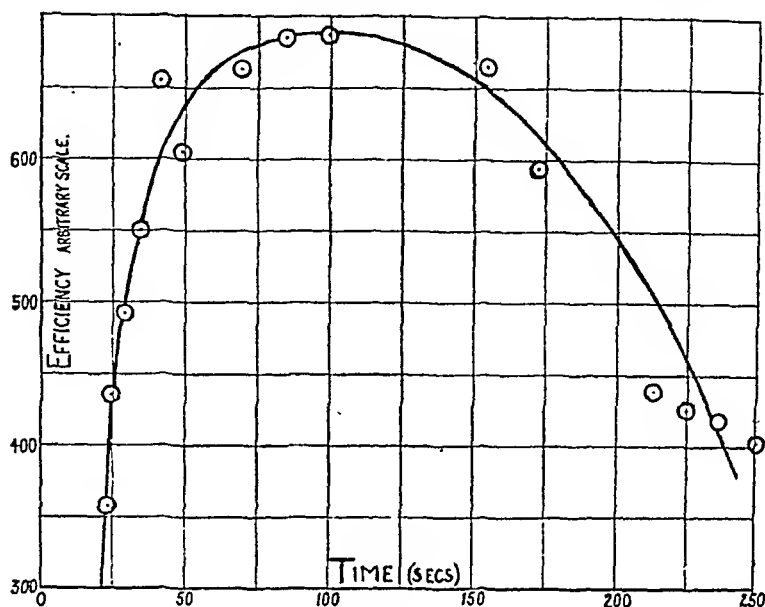


Fig. 1.

work") becomes preponderant the efficiency slowly decreases. It will be seen that the curve is similar in form to that given by Hill (1) p. 328).

In all the experiments the vertical height through which the body was raised was 12.2 metres. In the particular experiment recorded in Table I the mass raised was 62 kilos. Hence the external work performed was 775 kgm.m. This is equivalent to 1772 gm. cals. The average respiratory quotient over the whole period was 0.83. From Zuntz and Schumberg's tables 1172 gm. cals. are equivalent at this respiratory quotient to 355 c.c. of oxygen. Table I shows that at the optimum speed of working the  $O_2$ -consumption required to perform this external work was 1456 c.c. Hence the maximum efficiency has a value of  $355/1456$ , i.e. of 24.4 p.c. This is in good agreement with values of the efficiency obtained by previous observers in other cases of human muscular movement. Thus Reach (6) in Durig's laboratory found values of 27.9 p.c., 27.2 p.c. and 24.1 p.c. for the maximum efficiency of turning a winch using the arm muscles; Benedict and Cathcart (7) in bicycle ergometer experiments found values of 24 p.c. and 25 p.c.; Campbell, Douglas, and Hobson (8), also using a bicycle ergometer, found values of 24.2 p.c. and 25.4 p.c.

The duration of a single contraction when the efficiency is a maximum can be obtained from Table I. The optimum time taken to mount 78 steps is 100 secs. Consequently the duration of a single contraction is 1.3 secs.

It is necessary to realise that the exercise of raising the body slowly against its own weight does not allow maximal contractions to be made. Consequently we are here dealing with sub maximal efforts. With regard to such efforts Hill has shown on theoretical grounds ((2), p. 38) (a) that the efficiency is smaller than in maximal efforts occupying the same time, (b) that the maximum efficiency of a sub maximal effort occurs when the duration of the contraction is longer than in the case of a maximal one. The value therefore of the maximum efficiency found in the present experiments should be smaller than the efficiency of a maximal contraction occurring at the same speed. Further, the optimum speed in these experiments would no longer be the optimum speed for maximal contractions, so that the maximum efficiency of maximal contractions would actually be still greater than observed here.

Although the shape of the curve in Fig. 1 is similar to the theoretical curve given by Hill, a close comparison will show that in the mounting of stairs the efficiency for the slower speeds falls off rather more rapidly than theory demands. This can be accounted for on the supposition that not only is oxygen being used in the maintenance of tension in the muscles undergoing actual shortening, but that it is used also by other muscles which are in activity maintaining the posture and balance of the body as it ascends. In the slower movements the increased oxygen consumption thus caused appears to be an appreciable fraction of the whole, and the efficiency is correspondingly reduced. It seems then that this additional cause of energy expenditure masks to some extent the phenomena occurring in the group of muscles which are doing positive work, yet as it is a maintenance phenomenon, and acts therefore in the same direction as maintenance in the group of muscles concerned in the movement, its effect is merely to enhance the primary phenomena, thus causing a more rapid fall in the curve.

## PART II

In the experimental determination of the constants ( $W_0$ ,  $k$ ,  $A$  and  $B$ ) of Hill's efficiency equation, the movement investigated was the flexion of the elbow in the position described by Hill (2) and Lupton (3). It was assumed that  $k$  uniformly had the value 0.26. This is the value found previously by Lupton in healthy young men, as were all the subjects of the following experiments. The assumption of this value in the following cases is based on the fact that careful experiments for the determination of  $k$ , carried out on two subjects (see (3)) of similar age and build gave values of 0.260 and 0.264.  $W_0$ , the maximum realisable



state" to be attained, and a fore-period of maintenance of  $2\frac{1}{2}$  minutes was allowed to elapse before collection of the expired gases was begun; this occupied a further period of 2, 3, or 4 minutes of maintenance. The maintenance was carried out by all subjects at approximately the same angle, that is, the forearm made an angle of about  $120^\circ$  with the upper arm. This angle was kept constant by placing a horizontal rod in front of each wrist at the height required, and the forearm was flexed until the wrist just touched the rod. In each experiment six determinations of the gaseous exchange whilst maintaining the weight were made, each determination alternating with a determination of the resting gaseous exchange. To ensure that the latter quantity should remain as constant as possible, the subjects came to the laboratory without breakfast.

In an actual contraction with the ergometer the maintenance factor in the expenditure of energy should not theoretically be that at any particular angle, *e.g.*  $120^\circ$ , but an average, with respect to time, of the values throughout the range of movement. The contraction is maintained as the arm passes from fully extended to fully flexed, and it requires, for example, more energy to maintain a given force when the forearm and upper arm make  $60^\circ$  with one another than when they make  $120^\circ$ . Three experiments were performed on one subject to determine the effect on the energy expenditure of maintaining the same force at different angles of the forearm relative to the upper arm. Table II shows one of these experiments.

TABLE II. Subject H. L., post-absorptive condition; tension maintained by each arm 3.4 kilos; cords fastened round wrists.

(1) Experiment	(2) Oxygen per min. c.c.	(3) Carbon Dioxide per min. c.c.	(4) Angle	(5) O <sub>2</sub> per min. resulting from maintenance. c.c.
Resting (standing)	192	164	—	—
Maintaining (arms only)	202	172	$135^\circ$	—
Maintaining 3.4 kilos	222	182	120	20
" "	223	194	110	21
" "	234	198	90	32
" "	233	204	80	31
" "	243	218	70	41
" "	256	219	60	54

Column 5 in the table gives the differences between the O<sub>2</sub>-consumption maintaining the arms only and that maintaining the weight. These values therefore represent the energy required to maintain a tension of 3.4 kilos in each arm. The reason for the variation with angle is probably simple. The tension maintained by the whole arm at each angle is the same. As the muscle shortens, however, owing to the elastic properties of the fibres, each fibre must exert a smaller tension. Consequently, a larger number of fibres is brought into play and the oxygen consumption rises. The correct value to assume therefore for the maintenance energy is the average (with respect to time) of the values throughout the shortening. Assuming that the shortening occurs with a uniform acceleration, as is very nearly the case ((2), p. 34), it is obvious that the time-average will lay much more emphasis on the values in the extended, rather than on those in the flexed position. To Professor Hill I am indebted for a mathematical method of calculating from the above data the

average required, viz.,  $\frac{1}{t_0} \int_0^{t_0} B dt$ , where  $t_0$  is the total time occupied in the contraction. It is unnecessary to give this method of calculation here, but the conclusion is that the correct average value to assume is the same as that occurring when the forearm and upper arm make an angle of about  $120^\circ$  with one another. In the experiments on the oxygen consumption associated with the maintenance of a given force, this was the angle actually adopted, so that the single values obtained may be assumed to be close to the average value (with respect to time) in a uniformly accelerated contraction on the ergometer.

Table III gives  $W_0$  together with the results of the experiments on *setting up* isometric contractions.

TABLE III.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Subject	$W_0$ in single maximal contraction kgm m.	Max. T. in single maximal contraction kilos	Total T set up in metabolism exp kilos	Total excess $O_2$ in metabolism exp c.c.	$O_2$ per unit T. set up, c.c. col 5 - c.c. col 4	$O_2$ in setting up max. T col (8) x col (3) c.c.	A. energy used in setting up contraction capable of doing work $W_0$ kgm.m
W. B.	10.06	20.75	3470	2075	0.508	16.0	34.2
W. B.	10.06	20.75	3412	1900	0.557	14.0	31.8
A. S. C.	12.4	25.5	2954	1440	0.488	12.4	20.5
H. L.	6.38	21.65	3020	955	0.316	6.85	14.05

It can be calculated from Table III that the total energy used (col. 8) in *setting up* a contraction capable of doing maximum work  $W_0$  (col. 2) has the following values in the four experiments:  $3.1 W_0$ ,  $2.9 W_0$ ,  $2.14 W_0$ , and  $2.3 W_0$ . The mean value is  $2.6 W_0$ . In his original papers (1, 2) on the subject Hill assumed, in lieu of direct evidence, and on the basis of experiments on the isolated muscle of the frog, a value of  $2.5 W_0$ . This is, to all intents, identical with the mean value  $2.6 W_0$  found in man: which is striking evidence of the possibility of application to man of results obtained on isolated frog's muscle.

Table IV gives the results of maintenance experiments.

TABLE IV.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Subject	Mean $O_2$ consumption, maintaining arms only c.c. per min	Mean $O_2$ per min maintenance of 3.4 kilos by each arm c.c.	Max. T that can be developed and maintained kilos	Tension actually maintained kilos	$O_2$ for maintenance, per kilo per sec c.c.	$O_2$ for maintenance of max. T per sec. c.c.	B. energy used per sec. in maintaining max. pull kgm.hr
W. B.	$233 \pm 1$	$19 \pm 2$	26.75	3.4	.093	2.49	5.32
A. S. C.	$265 \pm 2$	$22 \pm 2$	25.5	3.4	.108	2.75	5.87
H. L.	$203 \pm 1$	$14 \pm 2$	21.65	3.4	.069	1.49	3.18

*Discussion of Results.* The experimentally determined values of the constants  $W_0$ ,  $A$  and  $B$  given in Tables III and IV, along with the

one arm. And for a quantity to have a 96 p.c. chance of lying within a range  $\pm 5$  c.c. of the mean value, instead of 25 observations, six only would be necessary.

In columns (2) and (3), Table IV, the probable errors of the means are given. Thus, for instance, in the case of the subject A. S. C. the values of the  $O_2$ -consumption maintaining the weights were 317, 305, 316, 300, 307, the values of the resting  $O_2$ -consumption being 264, 271, 262, 263 c.c. per min. It may be concluded therefore that the differences produced by maintenance in these experiments lie well beyond the limits of experimental error, and may be taken therefore as representing truly the quantities under consideration.

### SUMMARY.

1. In the group of muscles used in mounting stairs, in so far as they can be isolated from other muscle groups involved in the maintenance of the posture of the body, the mechanical efficiency of movement varies with speed in a manner analogous to that predicted by A. V. Hill on the basis of experiments on the flexors of the elbow and on isolated frog's muscle. There is an optimum speed of movement at which the efficiency is a maximum. This maximum efficiency has a value of about 24 p.c., and occurs when a single movement of the leg occupies about 1.3 secs.

2. A direct determination has been made, on the flexors of the elbow in man, of the constants involved in Hill's theoretical equation for the efficiency of muscular movement. Substituting these experimental values, (i) the calculated maximum efficiency agrees well with that directly observed on man (a) in this investigation, and (b) by previous experimenters: and (ii) the calculated optimum duration agrees well with the value directly observed. This is regarded as strong evidence for the validity of the theory underlying Hill's efficiency equation.

3. The energy required in the flexor muscles of the human arm *to set up* (as distinguished from *to maintain*) a contraction capable (if maintained) of doing maximum work  $W_0$ , is about  $2.6 W_0$ . This is close to the value  $2.5 W_0$  deduced by Hill from experiments on frogs, and gives a theoretical maximum efficiency of  $38\frac{1}{2}$  p.c. The energy required to maintain the contraction, if it is actually to do work, is one factor in diminishing the efficiency: the other factor is the irreversible frictional loss involved in an actual contraction at a finite speed.

My thanks are due to Professor A. V. Hill for his interest and help throughout the investigation, to Mr C. N. H. Long for acting as observer

in certain experiments, and to all who submitted themselves as subjects, to my often exacting demands.

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## APPENDIX

A note on certain critical observations by T. E. HANSEN  
and J. LINDHARD. BY A. V. HILL.

IN a recent paper (*This Journal*, 57, p. 287, 1923) Hansen and Lindhard have published certain criticisms of the principles underlying the equation  $W = W_0 \{1 - (k/t)\}$ , relating the maximum work to the duration of the contraction. While appreciating the value of their criticisms, and the friendly way in which Prof. Lindhard submitted his conclusions to me before publication, I feel that it is necessary, to prevent misunderstanding, to point out that—assuming their correctness—they have nevertheless no bearing whatever on the validity of the methods used, and the results obtained, by Lupton in the preceding paper. In the first place the equation does, in point of fact, fit the observations of  $W$  and  $t$  with great accuracy, provided  $W_0$  and  $k$  be suitable constants. This is shown not only by my original experiments, and the more accurate subsequent ones of Lupton(3), but by those of Hansen and Lindhard themselves. Lupton's experiments, employing (unlike Hansen and Lindhard's) automatic recording of the time  $t$  and a quick release mechanism, were made (as were mine) over a part of the curve, relating  $W$  to  $t$ , which is a far better test of the validity of the equation than the straight part chosen by Hansen and Lindhard: and the agreement was, as an empirical fact, complete. Taking due account of the greater errors necessarily associated with measuring short times, of the order of 1 sec., with a stop-watch, the agreement of Hansen and

Lindhard's observations with the equation is almost equally good, as the following calculations show.

(i) Observations on E. H.  $W = 12 \{1 - (0.196/t)\}$ .

Time	...	0.5	0.625	0.74	0.97	1.47	2.17	5.03
Calculated $W$		7.3	8.2	8.8	9.6	10.4	10.9	11.5
Observed $W$		7.4	7.75	8.6	9.8	10.4	11.0	11.5

(ii) Observations on J. L.  $W = 14 \{1 - (0.264/t)\}$ .

Time	...	0.54	0.62	0.80	1.07	1.5	2.4	5.15
Calculated $W$		7.1	8.1	9.35	10.5	11.5	12.5	13.3
Observed $W$		7.8	8.5	9.2	10.4	11.4	12.5	13.9

If the interpretation of  $W_0$  as the theoretical maximum work be pressed, and if  $W_0$  be then determined by an independent method and not from the observations, it is admitted that the equation fails: this fact will be considered later. For the moment, however, we may consider  $W_0$  and  $k$  as empirical constants, in which case the equation represents the observed relation between  $W$  and  $t$  with almost complete accuracy.

In Lupton's paper  $W_0$  and  $k$  are determined from observations with the ergometer, and the equation therefore may be assumed to give a true expression of the facts. Lupton next determines  $A$ , the energy required to set up maximum tension in the muscles employed, by measuring the oxygen used in setting up (but not maintaining) a series of isometric contractions, and multiplying this by the ratio (maximum tension)  $\div$  (total tension actually set up). He then determines  $B$ , the energy required to maintain maximum tension for unit time, by measuring the oxygen used per second in maintaining a submaximal contraction (sufficiently small for a "steady state" to be attained), and multiplying this by the ratio (maximum tension)  $\div$  (actual tension maintained). In the equation therefore for the efficiency

$$E = \frac{W}{H} = \frac{W_0 \{1 - (k/t)\}}{A + Bt}$$

the numerator represents the *actual* work done in a maximal contraction occupying time  $t$ , the denominator the *total* energy used in setting up a maximal contraction and in maintaining it for the time  $t$  necessary to allow the movement to be completed. Lupton, in the essential argument of his paper, makes no assumption that  $W_0$  and  $k$  have any particular theoretical significance, but only—what can be demonstrated empirically beyond dispute—that the equation  $W = W_0 \{1 - (k/t)\}$  in which they occur does actually represent the work done in a contraction started, by means of the quick release mechanism, after maximum tension is developed. The expression for the total energy,  $H = A + Bt$ , rests on

evidence (Hartree and Hill<sup>(4)</sup>) which Hansen and Lindhard do not deny, so that the expression for the efficiency does represent the actual work done by the muscles involved, divided by the total energy used by them in doing it. Its maximum value is then calculated by Lupton and found to agree well with the maximum efficiency found in actual experiments with the whole body. Lupton's methods and results therefore are completely unaffected by the criticisms of Hansen and Lindhard.

In their paper Hansen and Lindhard attempt to measure the efficiency directly, in a maximal pull on the inertia ergometer. It is obvious from their results, as was to be expected, that the efficiency of the actual muscles involved in doing the work is masked by the energy liberated in the static effort of other muscles. Indeed the oxygen consumption due to the static effort of other muscles was clearly about ten times as great as that of the muscles carrying out the actual pull on the ergometer. This source of error was carefully eliminated by Lupton by balancing the pull of the two arms one against the other, by using submaximal efforts in the oxygen measurements, and by similar means. The fact that Hansen and Lindhard obtained such low values (about 2 p.c.) for the efficiency, by an obviously and admittedly imperfect method, is no proof that reliable figures cannot be obtained, for a similar movement, by a more adequate one.

The disagreement between Hansen and Lindhard on the one hand, and Lupton and myself on the other, arises from the following cause: They attempt to calculate  $W_0$ , the theoretical maximum work, from an "indicator diagram" (maximum force against length of contraction) in the manner adopted by Fick and myself on frog's muscle; we, from observations of actual work on the inertia ergometer. The essential point is *not*, as they assume, whether the equation  $W = W_0 \{1 - (k/t)\}$  is valid or not, *but why the value of  $W_0$  calculated from the indicator diagram is some 10 p.c. to 20 p.c. greater than that determined from the actual work experiments* (14.1 kilogram metres instead of 12; 15.3 instead of 14). This is not a very startling disagreement, but it does seem to exist, and its existence requires consideration. It may depend, as Hansen and Lindhard suggest, upon some kind of fatigue effect arising in the maximal contractions of longer duration. Apart from any equation, the value of  $W$  observed does not, as the duration of the maximal pull is increased, approach asymptotically the value  $W_0$  calculated from the indicator diagram. This is obvious enough simply on plotting Hansen and Lindhard's results, and the essential (and

important) point which they have discovered is that the actual work always remains some 10 p.c. to 20 p.c. less than the theoretical maximum. A discussion of the equation in this connection, with a proof that  $k$  is not constant when calculated from an *inappropriate* value of  $W_0$ , tends only to confuse the issue.

Hansen and Lindhard credit the disagreement to fatigue of the muscle fibre, affecting the longer maximal contractions more than the shorter ones. This undoubtedly would produce the effect shown, in which case  $W_0$  calculated (*i.e.* really extrapolated) from the observations with the ergometer would be somewhat smaller than the theoretical maximum, being the value attained asymptotically by the work, in a series of contractions of progressively increasing duration and therefore fatigue. They argue that "strong isometric contraction is a hindrance to the blood supply and thus hastens fatigue." As a matter of fact the phenomenon under discussion is shown quite well if  $W_0$  be calculated from the ergometer readings lasting not more than one or two seconds, and in a fresh muscle the average tension which it is possible to maintain for such a time is certainly not 10 p.c. to 20 p.c. less than the maximum tension from which the indicator diagram is calculated. If it appear to be so there are two possibilities, one mechanical and the other physiological: (*a*) that the dynamometer<sup>1</sup> has inertia and overshoots its true initial reading in a rapid contraction, or (*b*) that during an actual movement, however slow, against an object which actually "gives," a certain amount of nervous inhibition is produced reflexly, from joints, muscles and tendons, and it is not possible to exert quite the same force as when the object is absolutely immovable. A fresh muscle is not fatigued in two seconds, neither does Wedensky inhibition occur at the nerve ending so rapidly, and certainly the absence of blood supply cannot affect it appreciably in such a time, since the oxidative recovery process takes minutes and not seconds. Moreover, if the quick release mechanism of the ergometer be not released immediately after the development of maximum tension, but delayed by a second or two, there is no appreciable diminution in the work. If fatigue came on rapidly, delayed contractions should give noticeably less work. I cannot agree with Hansen and Lindhard therefore in attributing the disagreement to fatigue, except possibly in very long contractions. Apart from a purely

<sup>1</sup> No proof that this criticism does not apply has been given by Hansen and Lindhard: the observations of the tension should be made on a revolving drum (so as to detect vibrations) by a tension-lever of high natural frequency, similar to that employed by Lupton. With a suitable lever fatigue does *not* manifest itself within 0.1 sec.

mechanical explanation it would seem to me far more likely that the actual process of shortening, causing movement of muscles and joints, produces a small reflex withdrawal of innervation from some of the muscle fibres, which is not produced when the contraction remains rigidly isometric.

Prof. Stopford does not concur in the suggestion that he has misled me, in the matter of the flexors of the elbow. As regards, however, the fundamental point at issue, viz. the variation of work and efficiency with speed, and the fact that the work calculated from the indicator diagram is unattainable in practice, the question of which muscles are actually involved in doing it is of little importance and need not be further discussed.

In view of the above, I feel that the somewhat stern dismissal of the equation  $W = W_0 \{1 - (k/t)\}$ , in the Summary of Hansen and Lindhard's paper, must be taken not as representing its incompetence to describe the facts, but rather its inability to reconcile the facts with a particular interpretation advanced by Hansen and Lindhard.



respiratory record though valuable when an exclusively inspiratory tracing is required.

*Experimental.* The previous communication emphasised the existence of a group of nerve cells (apneustic centre) located at the level of the striæ acousticæ which, when cut off from above, gives rise to prolonged tonic contraction of the inspiratory muscles. In the experiments described the tonus was obviously brought to an end by deoxygenation of the blood. The lack of  $O_2$  stimulated the lowest medullary centre sufficiently to occasion a few gasps. These re-oxygenated the blood and revived the striæ region, and a fresh tonus (apneusis) resulted.

In a recent series of 27 experiments asphyxial conditions were prevented by continuous ventilation of the lungs. The chest was laid widely open by removal of the costal cartilages and sternum from the 3rd to the 6th rib. Different mixtures of  $O_2$  and  $N_2$ , to which sometimes a varying percentage of  $CO_2$  was added, were forced from a large rubber bag through a tracheal cannula into the lungs, exits being provided by snipping the edges of all the pulmonary lobes. By this means the functions of the different centres and the effects of the respiration of various gases could be studied uncomplicated by asphyxial conditions due directly to the unsatisfactory respiratory movements which might be occurring as the result of section of the brain stem, etc.

It was found that the inspiratory tonus could be made to last 30 minutes or more, and even then, only ending as the result of vagal stimulation, or other experimental interference. Fig. 1 from a cat whose brain stem had been severed at the mid-pons illustrates some of the results obtained in this class of experiments. The lungs were perfused with an atmosphere composed of  $O_2$  60 p.c.,  $N_2$  40 p.c. and the vagi were intact, but similar results are obtained also after vagotomy. The top tracing is from a tambour placed on the chest, the middle tracing is from a similar tambour on the abdomen below the level of the ribs, and the lowest tracing is the blood-pressure, the zero of pressure being 25 mm. below the 5 second time tracing. Except when intentionally inhibited by electrical stimulation of the vagus, the inspiratory tonus lasted for some 17 minutes at a time, until indeed from gradual blocking of the exit openings the ventilation became unsatisfactory. If, during this period the trigeminus or vagus was stimulated by blowing air into the nostril or larynx, or electrically, the apneusis was instantly inhibited (Fig. 1). If this was done soon after the apneusis had commenced, the moment stimulation ceased the inhibition was removed and the inspiratory tonus was resumed. It was only necessary to periodically stimulate the 5th

or 10th nerve, or if cut, their central ends, as soon as apneusis had resumed, to simulate the natural respiratory rhythm (Fig. 1). When,

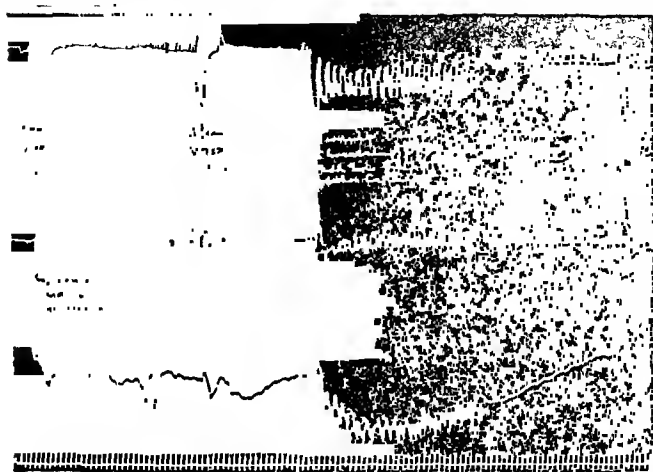


Fig. 1. Cat. See text. Continuous ventilation with  $O_2$  60 p.c. Prolonged inspiratory tonus inhibited periodically by vagal stimulation to simulate respiration of normal rhythmical type. All tracings read from left to right and time in 5 secs. Inspiration is upwards.

on the other hand, inhibition of apneusis was effected at a moment when the apneusis was about to terminate naturally, *i.e.* when the apneustic centre was fatigued or failing, the inspiratory position was not at once resumed but instead an active expiratory spasm occurred (Fig. 1).

The method of recording the respiratory tracings by tambours on the chest and abdomen is not ideal, and some explanation is necessary in order to interpret the records. The tambours, bound to the animal's body, are each connected with recording tambours, which must be arranged to write synchronously. On examining the thoracic tracing it is seen that inspiration writes upwards, expiration downwards. There is, however, one exception; if diaphragmatic relaxation or active abdominal expiration occurs so rapidly that the air cannot get out of the trachea quickly enough, then for an instant the thorax is distended and the writing

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By comparing the synchronous tracings of the thoracic and abdominal tambours, and by visual and tactile observation the correctness of this



Fig. 3. Cat. Vagus cut. Thoracic (top) tracing—expiration as a depression below the base line. Abdominal (2nd) tracing—expiration as a rounded rise between, and at one period higher than, the inspiratory rises. Active expiration at first thoracic spreads later to the abdominal muscles soon causing fatigue. Third tracing = B.-Pr. Zero 33 mm. below the 5 sec. time tracing.

interpretation was apparent. During respiration of the apneustic type these events occur so deliberately that it is easy to study and understand them (Figs. 1 and 2). Purely expiratory spasms show on the abdominal tracing alone, but when they merge as they sometimes do into convulsions these show on both thoracic and abdominal tracings. This forms a ready and reliable means of differentiating these two processes (Fig. 4).

Another series of experiments confirming the results previously published is illustrated in Fig. 5. Tracings 1 and 2 are from different cats. They show the effects of shutting off the blood supply from the brain and its stem by ligature of both carotids and subsequent compression of both vertebrals. About half a minute after the blood is completely shut off the pneumotaxic centre fails, respiration becomes slow and then

apneustic in type, a long inspiratory tonus is followed by a few short failing apneuses. Very soon gasps alone occur and death results. If,

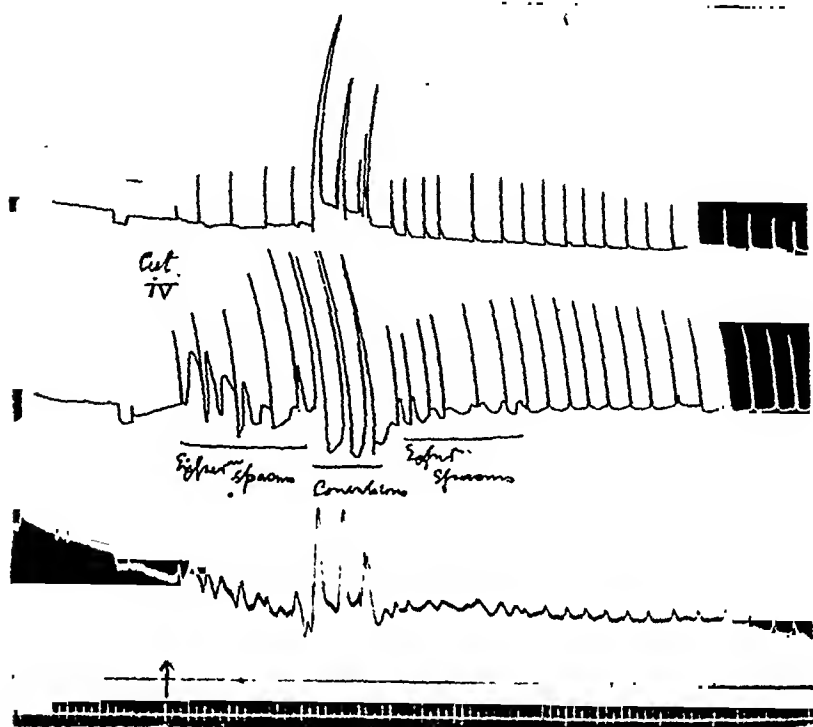


Fig. 4. Cat. Cerebellum removed, the vagi cut and the brain stem severed at level III. At the arrow a further section of the medulla just below the striæ acousticae (level IV). Apneuses instantly cease. Gasps and expiratory spasms continue, the latter at one stage pass into convulsions and only then show on the top (thoracic) tracing, while all spasms show on the middle (abdominal) tracing. Independence of the rhythms of gasping and expiration.

however, as in the experiment of which Fig. 5 is a record, the vertebrals are freed before gasping ceases, recovery takes place in the reverse order. Gasps give place to short and then to long apneuses; by periodical inhibition of these, slow respiration and soon normal breathing result. Active expiratory effects are usually not seen in such experiments on account of the rapidity with which the whole striæ region dies. In Fig. 5 the lowest line of tracings shows at 3 the failure of pneumotaxis from gradually increasing anæmia of the brain stem. It will be noted that the attempts to inhibit inspiratory tonus get progressively weaker till well marked apneuses appear. At 4 the reverse process is seen during recovery

from operative shock; here, apneuses are inhibited more and more successfully till normal breathing is resumed.

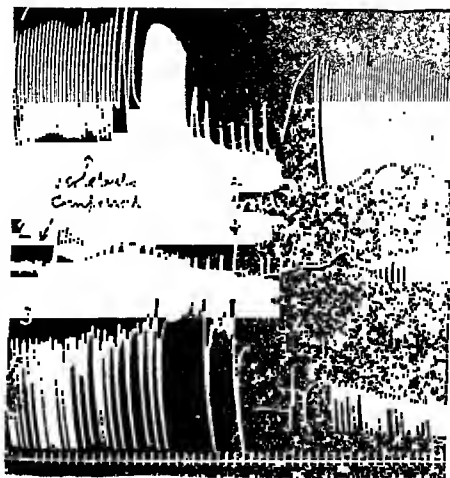


Fig. 5. See Text. Tracings 1 and 2—respiratory failure from anæmia and recovery. 3—gradual failure of pneumotaxis. 4—gradual recovery. 1 and 3 are from one cat, 2 and 4 from different cats.

Both these classes of experiments suggest that the periodicity or rhythm of respiration is normally produced by periodical inhibition of apneusis, supplemented in all but the quietest breathing by stimulation of the expiratory centre. It follows that rapid respiration is an evidence of expiratory activity and not as has been assumed an inspiratory sign. Thus quick breathing must not, for instance when it follows vagal stimulation, be regarded as a proof that the vagi contain inspiratory fibres.

*Localisation of the expiratory centre.* In a great many experiments in which section of the pons had been carried out it was noticed that when the central nervous system (and with it the various respiratory centres) were dying *gradually* from above downwards, expiratory phenomena invariably outlasted all evidences of the activity of the apneustic centre. When respiratory failure is *rapid* the apneustic and expiratory

centres usually die simultaneously so that in this case no expiratory phase is seen after apneustic failure (Fig. 5). After complete gradual apneustic failure active expiratory spasms continued along with gasping but that these two processes were due to distinct mechanisms was evidenced by the fact that they occurred neither synchronously nor with any regular relation one to the other. Thus the gasps may occur at any phase of the expiratory spasms during their onset, their height, or their recession (Figs. 4 and 6). After a short time (5 to 15 mins.) the expiratory efforts gradually fail and typical spasmodic and purely inspiratory gasping continues alone.

These observations suggested that either the expiratory mechanism was more resistant than the apneustic mechanism or that the former was placed at a lower level in the brain stem. Now it is found that the expiratory mechanism tires readily when constantly stimulated, *e.g.* when  $\text{CO}_2$  is in excess (Figs. 2 and 3), while inspiratory tonus is practically tireless as long as the apneustic centre is healthy. It was thus probable that the expiratory centre was not the more resistant of the two and that it would be found to lie below the apneustic centre but above the gasping centre.

Eleven experiments were performed to locate the centre. The method used was that described in my former article up to the point when trephining had been effected. Thereafter most of the occipital bone was removed, hæmorrhage was controlled by local pressure or by pushing wax into the cut surfaces of the bones. The cerebellum was split along the middle line and its peduncles were severed. It was then removed and the brain stem thus exposed was divided just above the striæ (crucial level III). The respiration became immediately of the apneustic type. A further section was next made just below the striæ (level IV), inspiratory tonus at once permanently ceased, and instead powerful tetanic expiratory spasms interspersed with gasps occurred (Fig. 4).

Unfortunately this latter section so seriously interfered with the blood supply to the medulla immediately below that the type of respiration invariably evoked was not long maintained. The expiratory spasms increased in severity for a minute or two (*cf.* Figs. 2, 3) and reached a climax in which they became clonic and spread as a convulsion widely over the body and even to the limbs. Soon the intensity of the spasms diminished again and in five or six minutes they ceased; the gasping continuing alone. It was noticeable (Figs. 4, 6) that when active expirations and gasping went on concurrently they did so asynchronously each at its own independent rhythm. In some cases before the expiratory

spasms had ceased a further section at crucial level V was made. This at once stopped the spasms, gasping continuing alone. Hemisection at

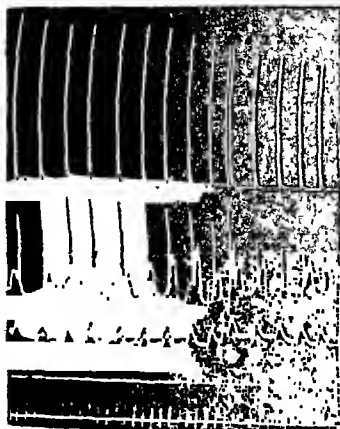


Fig. 6. Cat. Brain stem out just below the striæ (level IV). Gasping and expiratory spasms occur but are neither synchronous nor correlated. Top tracing, thoracic; middle, abdominal; lower, B.-Pr.

level VI near the apex of the calamus halves the height of the gasps, completing this section causes their immediate cessation and death rapidly supervenes.

These experiments confirm the facts observed in animals whose brain stem is slowly dying from above downwards. They indicate that a special centre for active expiration does exist and that it is placed immediately below the level of the striæ acusticæ but well above the gasping centre or *nœud vital*.

A number of experiments have been carried out in order to show the specific effect on each of the centres, of various afferent nervous impulses and of chemical changes in the blood circulating through them. It is proposed to report later the results obtained.

### Discussion.

In describing their observations on the respiration of decerebrate cats, Trevan and Boock(5) mentioned that sometimes the thoracic and



abdominal inspiratory rhythms appeared to be dissociated in time, the thorax rising first and later the abdomen. I think it probable that they mistook the expiratory protrusion of the abdomen for an inspiratory effect. I was myself guilty of this mistake until by taking a tracing direct from an expiratory muscle (external oblique) I learnt, correctly to interpret, the tracings from the abdominal parietes. A glance at Figs. 2 and 3 will show how easily such a mistake may arise. The fact is, however, that diaphragmatic and thoracic inspiratory movements are in all cases synchronous. This is what we would expect since both occur in response to efferent impulses leaving the same (apneustic) inspiratory centre.

Meyer and Meltzer (7) reported that the innermost abdominal muscle in chickens, which is purely expiratory, contracts regularly with each expiration. When the vagus is stimulated the chest becomes quiescent in the inspiratory position; whilst the expiratory muscle remains completely relaxed. This, they pointed out, was an instance of the general law of contrary inhibition or, as Sherrington calls it, reciprocal innervation. Head (8) had previously demonstrated the reverse condition, viz. that during expiration the diaphragm shows compensatory elongation. My own tracings taken separately from inspiratory and expiratory muscles in rabbits, and careful visual observation of the exposed diaphragm and abdominal parietal muscles in cats, confirm the above results. It may I think be concluded that during both inspiration and expiration the inspiratory and expiratory muscles oppose each other reciprocally by a continuous variation of tone upwards and downwards so that neither set of muscles is ever out of action for any length of time. But for such an arrangement each of the processes would be jerky and incoordinated. An example of unopposed inspiration is afforded by gasping such as occurs after section of the brain stem at level V. A sudden spasmodic inspiration is followed at once by collapse of the chest. The breath is not retained long enough to allow full interchange of the gases in the lungs. The process is hence quite unsatisfactory as a permanent respiratory method and has little similarity to controlled normal respiration.

It is certain that the expiratory centre acts quite independently of the gasping centre and it is very doubtful whether even the apneustic centre has any relation to the gasping centre except in the direction of inhibition. My view is that gasping is a relic of some transitory primitive respiratory process half way between gill and lung respiration as a fish gasps when taken out of the water. Yet I have seen so many instances in which gasping has been sufficient to revive animals whose higher

respiratory centres have temporarily failed that I feel no surprise that the faculty has persisted in the evolutionary struggle.

From watching the breathing of sea-lions I find that even when on shore their respiration is of a pronounced apneustic type. Periods of prolonged inspiratory tonus are separated by one powerful sudden expiration. When a scal rises for air a sudden violent expiration almost like a cough is instantly followed by a fresh apneusis and the animal sinks again for it may be some minutes.

Tortoises, turtles, alligators and crocodiles sometimes remain under water for an hour at a time and even when on land they retain each breath for 5 to 20 minutes. At the end of this pause the first movement is expiratory. One or two slow breaths may be taken and expelled, a final inspiration then occurs, the nostrils now close until after some minutes expiration determines the onset of the next respiratory cycle. Here, as in mammals, it is the incidence of expiration which settles the respiratory rate by terminating more or less frequently the inspiratory tonus which is in reptiles the position maintained during the prolonged respiratory pause. The reptilian type of breathing is thus purely apneustic and such as occurs in a cat whose pneumotaxic centre has been removed and in which continuous ventilation prevents the rapid onset of asphyxial conditions.

While this apneustic type of breathing suits excellently animals of amphibious habits, it is neither satisfactory nor necessary in the case of purely terrestrial beings. Hence I suggest arose the call for a higher centre automatically and periodically to inhibit apneuses and so produce respiration of normal type. Such is clearly the function of the pneumotaxic centre in the upper part of the pons, for when it is eliminated, e.g. by section (at level II), true-rhythmical respiration no longer occurs. In rabbits, this centre is less indispensable than it is in cats, monkeys, and probably in man. To some extent a dominant vagus replaces in rabbits the pneumotaxic centre. In these animals removal of the pneumotaxic centre alone does not stop rhythmical respiration, neither does vagotomy if the pontine centre is uninjured, but if the pneumotaxic centre and the vagi are both put out of action then apneustic respiration becomes at once fully developed, and is in every way comparable to that produced in cats and monkeys by destruction of the pneumotaxic centre alone (see Fig. 2). This confirms the results described by Rosenthal(6), Marckwald(2) and others in rabbits.

Expiration during quiet respiration is largely passive and is probably managed by simple inhibition of the apneustic centre by the automatic

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## PULMONARY ŒDEMA AND CONGESTION IN THE HEART-LUNG PREPARATION. BY D. T. BARRY.

*(From the Institut Marey, Boulogne-sur-Seine  
and University College, Cork.)*

ONE's first efforts with the heart-lung preparation of Starling are beset with many difficulties. The most troublesome incident is the occurrence of œdema of the lungs which comes on sometimes quite suddenly even when one thinks that the conditions are not favourable to it.

Knowlton and Starling<sup>(5)</sup> ascribe the condition chiefly to overfilling of the pulmonary vessels from the venous reservoir. Now Patterson, Piper and Starling<sup>(8)</sup> have shown that the heart adapts itself with apparent ease to great increase of venous inflow, up to 2500 c.c. per min. with a heart of 56 grams, and pumps the blood freely through the arterial system as long as there are no undue obstacles to output. Accordingly, though the pulmonary vessels be dilated to accommodate an extra amount of blood when the venous flow is increased, we should expect no very serious engorgement or obstruction of pulmonary flow until the heart fails to cope with the increased supply. As Starling<sup>(10)</sup> has shown, there can be little exudation until the pressure in the small vessels exceeds the osmotic pressure of the proteins in the blood. With pressures of 10 to 12 cm. of water in the right auricle it is probable that the pressure in the arterioles of the lung would not be much above 30 mm. Hg., thus permitting an insignificant filtration pressure with the colloid concentration of normal blood. In the writer's experiments the lungs have withstood œdema for long periods with this venous pressure, that is, they have been freely flushed with blood containing a suitable amount of colloids without the occurrence of œdema unless arterial obstruction or valve lesion supervened. Congestion usually precedes œdema in these latter conditions. Chillingworth and Hopkins<sup>(4)</sup>, working with an animal in a plethysmograph, were able to occlude completely the pulmonary vessels so that no blood passed through to the left side of the heart. The maximum pressure attained in the pulmonary artery was 80 mm. Hg. Probably that in the arterioles was 30 mm. less, and with incomplete occlusion though still with considerable resistance it would be further reduced by 15 or 20 mm. Hg.

When the blood was but slightly diluted below the standard to be given presently it was noticed, when œdema set in, that there was a difference between the amount of blood put out on the arterial side and the possible intake as marked by the venous tap. That is to say, the blood was held up in the lungs. In some instances, with a moderate flow and appreciable dilution, œdema occurred when there was practically no difference between output and amount let in. This means that exudation occurs with diluted blood independently of obstruction and of amount of flow.

Another factor in exudation which is supposed to be important is increased permeability of the vessel walls. The stretching of the lung vessels should not cause this, for otherwise, there must be risk of pulmonary œdema with any sustained increase of venous pressure in great physical efforts. It is a question whether the pulmonary vessels lose tone and resistance when exposed, thus becoming more permeable. It would seem to be implied by Knowlton and Starling<sup>(6)</sup> that they do, as the occurrence of œdema in natural conditions must require something more than the increased venous flow to which they refer. The lungs can resist the mere exposure for long periods, since in the natural conditions of the circulation, with the chest open, the lungs show greater resistance than with the artificial conditions of the heart-lung preparation. The exudation into the alveoli and bronchioles which occurs in addition to that into the interstitial tissue is predisposed to by excessive ventilation and cold air.

The troubles which the writer experienced with œdema occurred chiefly in connection with experimental valve-lesions. When the valve was kept incompetent for some time and compensation began to fail, œdema set in more readily. Mitral regurgitation was established through an opening cut in a gum elastic catheter about  $1\frac{1}{2}$  inches from the "eye." Inserted through the auricular appendix, the catheter was pushed down so that the lower end was in the ventricle and the extra opening in the auricle, while the upper end was plugged or connected to a manometer. In some recent experiments a glass tube with a plunger, and a similar opening has been used instead. When the plunger is drawn out past the opening in the auricle regurgitation sets in. This tube was suggested by that of Wiggers<sup>(11)</sup>, but his was inserted through the ventricle wall. The great advantage of the tube adopted in these experiments is that according to the size of the extra opening blown in the tube the volume of regurgitated blood can be varied.

An investigation of the condition of pulmonary œdema has been undertaken not merely with a view to prolonging the "life" of the heart-

A. Simple œdema was taken to be established when froth appeared in the tracheal tube. The frothing is sometimes slow in setting in and the pulmonary tissue may show pitting and defective entry of air long before it. These symptoms were taken as sufficient indication in most cases where remedial measures are reported as successful, but frothing was the general indication of onset with the higher dilutions. It is more reliable for giving a uniform standard of comparison.

B. Congestion was marked at the bases of the lungs; with it supervening on œdema the froth generally became red. The exudate contains hæmoglobin. Cannon, Hooper and Fraser<sup>(3)</sup> cite a similar result from increased concentration of salt in the fluid injected. It is necessary to state that although hypertonic saline was used in some of my experiments this sanious exudate also occurred with isotonic fluid.

In making membranes for ascertaining osmotic pressures I have sometimes set up one consisting of cloth impregnated with celloidin which proved to be easily permeable to hæmoglobin. A pressure of 15 or 20 mm. Hg was registered when blood was tried against Ringer's fluid with such a membrane, the pressure then falling back to zero with diffusion of hæmoglobin into the saline. When the blood was made hypertonic with sodium chloride the diffusion seemed to be better marked, but no accurate observations were made from this standpoint. Gum is much less diffusible, and this is a point of some significance if it be proposed to treat œdema with gum injections, especially toxic forms where there is increased permeability of the vessel walls and albumen in the exudate.

The permeability of the lung capillaries then permits the passage of hæmoglobin. When such an exudate accompanied congestion the two factors of low osmotic pressure and obstruction to output were generally well marked. Hæmolysis quickly sets in in defibrinated blood with the addition of a little Ringer's fluid. In defective osmometers, as already stated, hæmoglobin may pass out when the pressure is slightly raised within the system; this is most marked with whipped or diluted blood with some degree of laking. There is less diffusion of hæmoglobin with fresh, oxalated blood. In the condition of œdema the hæmoglobin is generally held back, but when congestion supervenes the exudate becomes sanious. One generally associates congestion with mechanical obstruction, without giving much attention to the composition of the blood. One may even think that a diminished viscosity would mean diminished friction and therefore less tendency to congestion, but the tone of the vessel walls seems to fail with the increased permeability, so that congestion is

facilitated, as well as œdema. In dogs which have been kept for some time in the kennels, without exercise, there is often some hypostasis of the lung to begin with. If the limits and degree of this are marked beforehand, it need not interfere with one's observations. It cannot be taken as an objection to the experiments, for such lungs function like the perfectly normal organs in ordinary conditions, and the normal lungs behave in exactly the same way as the affected one in abnormal conditions. Mere pigmentation is not to be mistaken for hypostasis.

C. Ecchymoses resulted when the back pressure was great enough to cause rupture of small blood vessels in scattered patches. Although the amount of obstruction necessary to produce them is considerable, it is somewhat less than was at first thought probable. Given this obstruction they come on very rapidly and they may be produced without the obstruction causing permanent injury to the heart.

The blood was carefully examined in each experiment. The osmotic pressure could not be taken with sufficient ease or speed to make it a routine practice with each observation, but it was estimated for representative samples from time to time. The specific gravity was taken regularly by means of a series of mixtures of chloroform and benzene varying from 1045 to 1058, verified and corrected from day to day. The specific gravity, taken alone, is of little value as an indication of the osmotic pressure and viscosity; they have to be considered in addition. Viscosity has been taken as the chief indication of quality in all these experiments although it does not bear a linear relationship to osmotic pressure. The corpuscle content was measured in many samples as was the oxygen capacity.

An analysis of the gum arabic used was made by my colleague, Prof. Dixon, to whom I beg to express my best thanks. It shows a little over 3 % of ash. This includes calcium, potassium, with scarcely a trace of sodium. The Ca and K are chiefly combined with organic acids. Taking a strong solution (1) of 11 % in Ringer's fluid and diluting it 10 %, 20 %, etc., we get the following figures for viscosity and specific gravity with this gum.

	Solution %	Viscosity	Sp. Gr.
1	11	3.8	1042
2	9.9	3.4	1038
3	8.8	3	1035
4	7.7	2.45	1031
5	6.6	2.10	1028
6	5.5	1.68	1024

For the pure "Turkey elect" gum employed by Bayliss(2) and used



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B. Congestion was marked at the bases of the lungs; with it supervening on œdema the froth generally became red. The exudate contains hæmoglobin. Cannon, Hooper and Fraser(3) cite a similar result from increased concentration of salt in the fluid injected. It is necessary to state that although hypertonic saline was used in some of my experiments this sanious exudate also occurred with isotonic fluid.

In making membranes for ascertaining osmotic pressures I have sometimes set up one consisting of cloth impregnated with celloidin which proved to be easily permeable to hæmoglobin. A pressure of 15 or 20 mm. Hg was registered when blood was tried against Ringer's fluid with such a membrane, the pressure then falling back to zero with diffusion of hæmoglobin into the saline. When the blood was made hypertonic with sodium chloride the diffusion seemed to be better marked, but no accurate observations were made from this standpoint. Gum is much less diffusible, and this is a point of some significance if it be proposed to treat œdema with gum injections, especially toxic forms where there is increased permeability of the vessel walls and albumen in the exudate.

The permeability of the lung capillaries then permits the passage of hæmoglobin. When such an exudate accompanied congestion the two factors of low osmotic pressure and obstruction to output were generally well marked. Hæmolysis quickly sets in in defibrinated blood with the addition of a little Ringer's fluid. In defective osmometers, as already stated, hæmoglobin may pass out when the pressure is slightly raised within the system; this is most marked with whipped or diluted blood with some degree of laking. There is less diffusion of hæmoglobin with fresh, oxalated blood. In the condition of œdema the hæmoglobin is generally held back, but when congestion supervenes the exudate becomes sanious. One generally associates congestion with mechanical obstruction, without giving much attention to the composition of the blood. One may even think that a diminished viscosity would mean diminished friction and therefore less tendency to congestion, but the tone of the vessel walls seems to fail with the increased permeability, so that congestion is

relieved by the injection of gum but this objection could not hold if the corpuscular margin were not too narrowed.

In some cases viscosity was raised by the addition of blood which had been allowed to clot, the clot being broken up and squeezed through a cloth. This has a high viscosity owing to clumping—up to 5—which may be shown by a wide bore viscosimeter (it quickly clogs a narrow bore). It possesses an advantage over gum only when the circulating fluid has become very dilute with great reduction of the hæmoglobin. Osmotic pressure is not raised by it in a corresponding measure to viscosity.

In some instances, work was tried with the dead preparation. This can be used for many observations as the blood can be pumped through the apparatus by squeezing the ventricles with the hand. Œdema comes on with dilution of the blood as in the living condition, and clears up on the addition of gum.

### RESULTS.

I. *Varying composition of the blood.* (a) Low inflow and arterial resistance (A.R.); (b) high inflow and A.R. The protocols will show what occurs with the changes. For the dilution of blood the quantity in circulation was estimated from the capacity of the apparatus, of the heart and lungs and the amount seen in the venous reservoir. Something more than half of the requisite quantity of saline was added at once to the reservoir and the rest gradually during a minute or so. If the total amount were immediately added the lungs would at first receive a greater dilution than intended. The dilution must be considered approximate. Some discrepancies in sp. gr., etc., from those of standard deviations will be noticed.

(a) *Exp. 1.* In this and other protocols + indicates pitting of the lung and defective entry of air, and ++ indicates simple œdema or congestion.

Saline %	Sp. Gr.	Viscosity	Corpuscles %	Œdema	Time mins.
10	1053	2.7	36	0	10
15	1050	2.4	33	+	10
20	1045	2.2	30	++	5

From this it may be stated that œdema becomes quickly established in the easiest working conditions with blood, the viscosity of which has been reduced by about a quarter. Of course, it is not the viscosity which matters so much as the colloid osmotic pressure, but it provides a fair indication of this pressure in the solutions used.

(a) *Exp. 2.*

Saline %	Sp. Gr.	Viscosity	Corpuscles %	Œdema	Time mins.
10	1051	2.6	34	0	10
15	1048	2.4	32	+	10
20 and gum	1048	2.8	29	0	10

Here the œdema which began to appear with a viscosity of 2.4 disappeared when this was raised to 2.8, the last dilution was made with hypertonic saline (about 1.5 %) containing 10 % of gum.

(b) *Exp. 1.* Blood diluted nearly 20 %, plus a little gum, having sp. gr. 1050; viscosity - 2.5; corpuscles 25 %.

Inflow c.c.	A.R.	Œdema	Congestion	Ecchymoses	Time mins.
15	100	+	+	0	5
20	150	++	++	+	5

In this experiment congestion was seen early with a moderate flow and pressure. The exudate was sanious from the beginning so that congestion and œdema were practically simultaneous. Some 20 mins. from the setting up of the preparation, the heart was in fibrillation the blood being dark and thin; the heart had been beating apparently well on a 10 c.c. flow and A.R. = 60 when the amounts cited above were tried. The dearth of corpuscles in these solutions is but a small factor in the result. The other defects of the blood are chiefly responsible; the heart displays considerable resistance with similar percentages of corpuscles when viscosity is high and the lungs remain active.

Œdema and congestion are conditions of great moment when they supervene to complicate heart affections. Meakins(6) points to the rôle of the œdematous surface of the alveoli as a factor in oxygen desaturation with tachycardia. He suggests overcoming the difficulty by increasing oxygen pressure in the alveoli. From the results of the experiments now being described it seems that something more might be done than increasing oxygen pressure. Low blood viscosity was found in some cases of valvular disease of the heart, accompanied by engorged lungs and excessive bronchial discharge. In one of these, seen in conjunction with Dr M. Cagney, the oxalated blood drawn from a vein had a sp. gr. of 1054 and viscosity 2.7. The intravenous injection of gum to raise the colloid osmotic pressure in such cases should be of some service, although it is true that in the experiments it was only instances of mild œdema which cleared up fully when the osmotic pressure was increased.

II. *Blood of constant composition* (viscosity 2.6, corpuscles 30) with varying flow, A.R. and valve changes.

## Exp. 1.

Inflow c.c.	A.R.	M. valve	Œdema	Congestion	Ecchymoses	Time mins
15	100	Incompt.	?	0	0	5
"	120	"	+	+	0	5
25	180	"	++	++	+	5

With a flow and pressure equal to those with which congestion and ecchymoses were caused in this case, the heart succumbed in other cases before these could be set up in the lung. If the œdematous condition be in existence some time, of course the musculature suffers from the anoxæmia of the blood and fibrillation is to be expected with extra demands upon it. It is best to produce the defect in the valve beforehand and then to proceed quickly. It is compensated until the new factor disturbs it.

Wiggers<sup>(11)</sup> did not find that the lungs were prone to congestion as a result of a lesion of the mitral valve. He employed the heart with natural circulation. In this he was unable to produce any graduated increase of arterial pressure and used simple saline solution for increasing the venous flow but gave no definite figures for measurement of that flow. As he had no troubles to record from œdema of the lungs presumably he made but slight modifications of flow. Wiggers<sup>(12)</sup> objects to the heart-lung preparation for research of this sort on the grounds that it tells what can occur with changing conditions rather than what does occur with natural change. Exact measurements of intake and output are certainly an advantage in most forms of investigation, and it is only in the heart-lung that they can be made. For this reason, if for no other, the heart-lung must be considered a very desirable preparation.

There is no doubt that congestion of the lungs is more liable to occur when the valve is made defective than when it works efficiently, but congestion may come on without any lesion of the valve when the venous flow and arterial pressure are high. It is predisposed to by an œdematous condition, and by certain changes in the blood.

By pressure on the pulmonary vein at the root of one lung a one-sided congestion has been sometimes set up. This causes a great increase in size of the lung with gradual occlusion of air until no further expansion occurs with the inspiratory stroke. Slight exudation on the surface is noted after a time in some of these cases, with some pitting on pressure, but for a long time there is no true œdema if the composition of the blood be favourable. The gradual addition of saline to this causes the typical sanious exudate to appear in the cannula, that is, when the pressure on the vein is intermittent. Section shows this exudate in the alveoli and

vago-sympathetic nerve with vagal effect is invariably followed by a decrease of the surface tension of the perfusing salt solution, but that stimulation with sympathetic action is always followed by a marked increase of this tension, so that it rises to the value of the pure salt solution. Thus, the functional antagonism of vagus and sympathetic action on the heart is accompanied by a physico-chemical antagonism of the perfusing salt solution. As capillary active changes have a special importance for the permeability of the cell-membranes and so for the fundamental processes of excitation and inhibition, these results may have some interest for the general theories on the subject.

The methods used were very simple. The heart was perfused from the inferior lower vein *in situ*; the fluid, emerging from a cannula in the right aorta was collected in a watch glass and the surface tension measured at once. The venous cannula was inserted in the venous sinus; the hepatic veins and the left aorta were ligatured. The perfusion liquid was composed of NaCl .50 %,  $\text{NaHCO}_3$  .15 %,  $\text{CaCl}_2$  .6 aq .020 %, KCl .01 % and  $\text{CO}_2$  till  $\text{pH} = \pm 8$ . In this arrangement the heart normally survived for 2-3 days if the water used was distilled in Jena-glass vessels. As the surface tension of the emerging fluid was the factor to be measured, special attention has to be given to the absolute cleanliness of all objects which come in contact with the salt solution; the watch glasses must not be touched with the fingers, etc. The stimulation of the vago-sympathetic nerves was effected by means of electrodes in the Eustachian tubes. In the beginning of our researches the vagal effects always were the strongest; in later experiments the primary sympathetic effects became more intense. But generally there appeared in the curves a domination of vagal and sympathetic effects alternately, and the surface tension of the fluid pressed out varied accordingly.

Before describing our experiments with vagus and sympathetic excitation we must make a general remark on the surface tension of a salt solution which has just passed through the heart. Immediately after the beginning of the perfusion the tension of this pure salt solution (76-77 dynes per cm.) is considerably decreased by the washing away of the blood. If a slight lowering of the surface tension is to be traceable, it is necessary that the tension of the solution just leaving the aorta should be as high as possible, viz. the tension of a pure salt solution. The tension of pure water is 75 dynes, and of the salt solution 76-77 dynes per cm.

When the blood and plasma of the heart cavities are removed, the tension of the perfusing salt solution will rise for a very short time to its highest value of 76-77 dynes per cm. But this tension is very soon lowered by the presence of lipoids, which dissolve slowly from the cell-surface,

as was described by Clark in his work on the hypodynamic heart, and this is the condition in which the aortic solution remains for 4-7 hours after the beginning of the perfusion. Fig. 1 gives a general survey of the process described. After this period of washing away of lipoids, the surface tension is raised to the value of pure salt solution and so it remains; further, if no vagus excitation takes place, the heart next day becomes hypodynamic.



Fig 1

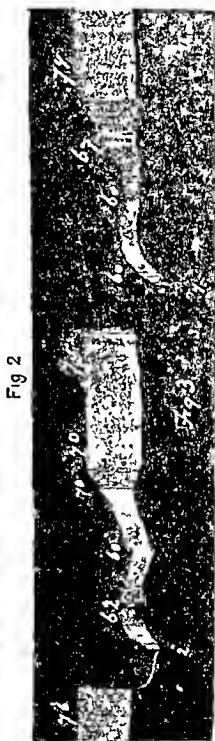


Fig 2

fig. 1. Perfusion lasting from 10 a.m. to 3 p.m. Tracing of heart-beat taken at approximately equal intervals. The number above each portion of the tracing gives the surface tension of the perfused fluid in dynes per cm.

fig. 2. Sympathectomic heart. Perfusion lasting from 2.30 till 6 p.m. There were trifling variations only in the surface tension of the omitted part of the curve.

sympathetic action does not disappear gradually but rather suddenly. This steep fall in the curve is accompanied by a small but distinct lowering of surface tension.

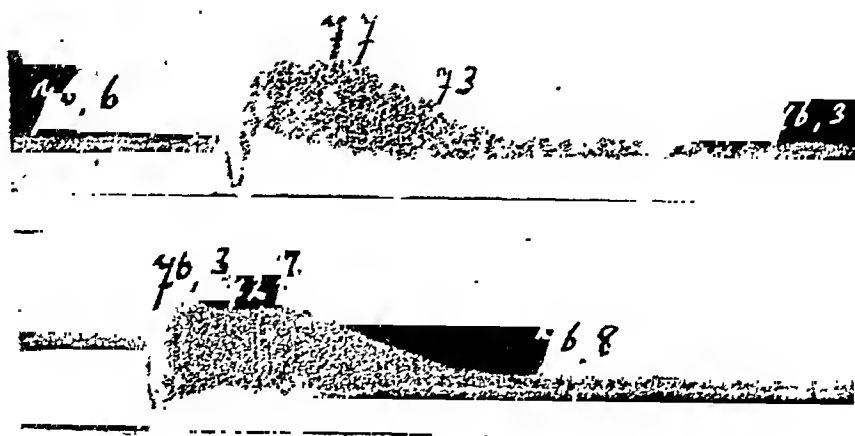


Fig. 7.

Finally, Fig. 8 shows the combination of vagal and sympathetic effect and the influence of the surface tension of the perfusing liquid. In this curve the auricular contractions are also visible. The heart had already been perfused for some hours and the capillary active substances were completely washed away. The first stimulation of the vago-sympathetic nerve at 1 caused a vagal inhibition which was not followed by a sympathetic after-effect (see also auricular contractions). In accordance with this process the surface tension decreased by the first stimulation does not rise again but remains lowered until the second stimulation, three hours later. This second stimulation had a primary sympathetic influence (at 2 and 3) and the surface tension rose to the water value for some moments. The then following disappearance of auricular contractions and the negative inotropic influence on ventricular contractions indicate the domination of a vagus after-effect (at 4) and the surface tension is decreased accordingly, till a sudden second outbreak of the sympathetic influences occurs (at 5) and the surface tension is raised at once to the value of a pure salt solution. The further stimulations followed the same cycle of alternating vagal and sympathetic effects with their reflection in the perfusing solution.

In this way we always found that a vagus influence is accompanied by decrease of surface tension of the perfusing saline, caused by the libera-

tion of some slightly capillary active substances and that a sympathetic action of the heart is at once reflected in the saline by a sudden rise of the tension.

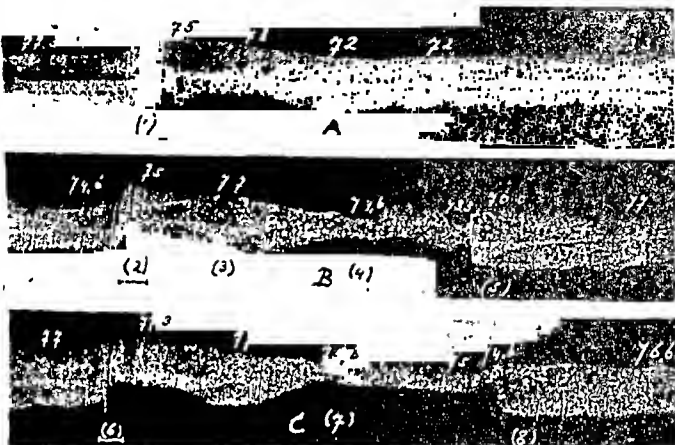


Fig. 8. A. The tension was 77.3 from 11.30 a.m. to 2 p.m., when the first tracing of the figure began. Weak vagus inhibition without sympathetic after-effect.  
B and C. Alternation of vagal and sympathetic influences.

We have made no pharmacological stimulation or inhibition, because it was our object to investigate the production of nerve-stimulating substances in conditions as physiological as possible. We believe we have demonstrated in a purely physico-chemical way that vagus-excitation is accompanied by production of an organic substance which can be detected in the perfusing saline and that sympathetic excitation is combined with a process by which the vagus substance is rendered capillary inactive, or prevented from solution in the liquid. In a further communication we hope to give a more definite analysis of these phenomena.

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# CARBON DIOXIDE TENSION AND OXYGEN CONSUMPTION DURING ARTIFICIAL RESPIRATION, ACIDOSIS AND ALKALOSIS.

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In a paper recently published (1) it was shown that injection<sup>1</sup> of air under the skin afforded a ready means to examine changes in  $\text{CO}_2$  partial pressure within the body; and also, that artificial respiration reduced the  $\text{CO}_2$  tension in air under the skin or in air in the abdominal cavity. In further observations very vigorous artificial respiration was employed; it was expected that the  $\text{CO}_2$  partial pressure within the body would be very rapidly and very markedly reduced thereby. On the contrary it was found that in most cases the reduction was fairly slow in rate and moderate in degree. It was therefore considered that the body was actively replacing the  $\text{CO}_2$  pumped out by the artificial respiration and the experiments described in the present paper give evidence in support of this hypothesis. The carbon dioxide tension in various regions of the body, the carbon dioxide expired and the oxygen consumption were estimated before and during artificial respiration and before and after intravenous injection of acid and alkali. In some cases the blood-pressure was recorded.

*Methods.* Air was injected under the skin by means of an ordinary hypodermic needle, left *in situ*, a small piece of rubber tubing closed by a clip being attached to the outer end of the needle. Escape of air was prevented partly by the elasticity of the skin and also by gently pinching the skin around the needle by means of another clip. Air was injected into the abdominal cavity by means of a glass cannula inserted during anaesthesia. Alveolar  $\text{CO}_2$  tension was estimated by the Higgins-Plesch method as used in the previous research (1) and the oxygen consumption by a slight modification of the Douglas-Haldane method of indirect calorimetry.  $\text{CO}_2$  content of blood was estimated by Van Slyke's method and the  $\text{CO}_2$  tension from curves obtained by equilibrations of samples of the blood of the same animal with known mixtures of air and  $\text{CO}_2$ . Artificial respiration was performed with Schuster's double action pump (2).

<sup>1</sup> When unanaesthetised cats were used, the injections were carried out by my colleague Dr W. E. Gye.

*The effects of artificial respiration on the carbon dioxide partial pressure in air under the skin and in the abdominal cavity.* Air was injected under the skin and into the abdominal cavity about a couple of hours before artificial respiration was commenced, in order that the  $\text{CO}_2$  tension in this air might attain equilibrium with that of the surrounding tissues. When the  $\text{CO}_2$  tension had become constant, artificial respiration was commenced. By this means air was pumped into and withdrawn from the lungs about 180 times per minute. Table I records the results. The total litres of air pumped into and out of the lungs per minute are given. If these figures be divided by 180, the amount of air pumped into and withdrawn by each stroke of the pump will be obtained; the average amount was 50 c.c. per stroke in the skin experiments and 60 c.c. in the abdominal cavity experiments. Artificial respiration at 8.9 litres per min. performed for 139 mins. reduced the  $\text{CO}_2$  tension in air under the skin on an average from 41 mm. to 30 mm.; whilst artificial respiration, at 11.1 litres per min., performed for 92 mins. reduced the  $\text{CO}_2$  partial pressure in air in the abdominal cavity on an average from 45 mm. to 20 mm. The lowest figure for the skin experiments was 21 mm., and for the abdominal cavity experiments 12 mm.

It seems probable that artificial respiration lowered the  $\text{CO}_2$  tension more rapidly and somewhat more markedly in air in the abdominal cavity than in air under the skin. Results published in the previous paper<sup>(1)</sup> indicated that under certain other conditions, *e.g.* increase of external temperature, the  $\text{CO}_2$  tension was higher in air under the skin than in air in the abdominal cavity.

During artificial respiration the  $\text{CO}_2$  tension was highest in the tissue spaces, *e.g.* abdominal cavity, whilst it was lower in the venous blood than in the tissue spaces, and lower in the arterial blood than in the venous blood and much lower in the pumped-out air than in the arterial blood. After 154 mins. of artificial respiration in Exp. 10 the  $\text{CO}_2$  partial pressure had fallen from 54 mm. to 12 mm. in the air in the abdominal cavity, whilst it was 10 in the venous blood of the heart, 7.5 in the arterial blood of the femoral artery and 1.1 in the air pumped out from the lungs. The air pumped out from the lungs could not be taken as a guide to the alveolar air. It was difficult to block up completely a bronchus during artificial respiration, but the results obtained from such attempts with a catheter showed that the  $\text{CO}_2$  tension in the alveolar air during pumping was at least from 3-5 times greater than that in the pumped out air. From these experiments it seemed evident that the reduction of  $\text{CO}_2$  tension in the tissues by artificial respiration was neither so great nor so

rapid as it was expected to be; the body was probably actively producing  $\text{CO}_2$  to replace the  $\text{CO}_2$  pumped out. To investigate this question the  $\text{CO}_2$  output and the  $\text{O}_2$  intake was estimated.

*Metabolism and blood-pressure before and during artificial respiration.* The metabolism, before artificial respiration, was estimated under anaesthesia by a slight modification of the Douglas-Haldane method. A small T-shaped tracheal cannula was fitted with respiratory valves designed by Lovatt Evans. The air was collected for from 10 to 20 mins. in a small Douglas bag, samples of air being analysed by the Haldane apparatus. Table II A gives the results obtained with cats under urethane; a small amount of ether was used during the operations for insertion of the tracheal and carotid artery cannulae. The average figures obtained for  $\text{CO}_2$  were 254 c.c. per kilo. and per hour and for  $\text{O}_2$  324 c.c. per kilo. and per hour.

The metabolism during artificial respiration was estimated by collecting the air pumped out from the lungs in an ordinary sized Douglas bag, connected by a rubber tube to the outlet pipe of the double-action pump; usually 50 litres of air were collected. It will be seen from Table II B that the average percentages for  $\text{CO}_2$  output and for  $\text{O}_2$  intake were very low, namely, on an average, 0.29 and 0.35 respectively. Because they were so low very great care was taken to be sure that they were correct. Four samples of air were analysed by two persons. The Haldane apparatus is known to be quite accurate enough to estimate such low percentages if due care be taken. The  $\text{CO}_2$  estimations by different persons were usually exactly the same in the present series. As the  $\text{O}_2$  estimations did not show quite such close agreement the lowest—not the average—figures obtained for  $\text{O}_2$  absorbed, were used in the calculations. Accurate analyses were also made of the laboratory air which was pumped into the lungs.

Table II B shows that during artificial respiration the average figures for  $\text{CO}_2$  output were 663 c.c. per kilo. and per hour, whilst the average  $\text{O}_2$  figures were 857. This average includes some figures from animals not included in Table II A. If only the averages for the same animals as in Table II A be taken, the figures for  $\text{CO}_2$  were 552 and for  $\text{O}_2$  719 c.c. per kilo. and per hour. It is thus evident that both the  $\text{CO}_2$  output and the  $\text{O}_2$  intake were over 100 p.c. higher during artificial respiration (Table II B) than before artificial respiration (Table II A). This indicates a marked effort on the part of the body cells to make good, by increased metabolism, the loss of  $\text{CO}_2$  during artificial respiration. The average amount of air pumped into and out of the lungs per minute was 10.6 litres, that is, about 60 c.c. per stroke at 180 strokes per min. In some cases the air

pumped into the lungs was warmed from room temperature, 16° C., to about 30° C.; the results for metabolism, however, were much the same at both temperatures, so that the degree of cooling of the blood of the lungs by the rapid pumping was not the main cause for the rise of metabolism.

Figures for blood-pressure before artificial respiration, are given in Table II A, whilst those for during and after artificial respiration are given in Table II B. It will be seen (Table II B), that in those cases where the metabolism was highest during artificial respiration, the blood-pressure recovered best after cessation of artificial respiration. On the other hand, when the metabolism was low, the blood-pressure did not recover well and the animal died. The blood-pressure tracings resembled very closely those published by Dale and Evans(3). In their research the duration of pumping was about 30 mins. They pointed out that the blood-pressure recovered well. In the present research in Exp. 5 (Table II B) pumping was carried out for 76 mins. and the blood-pressure recovered very well, the amount of air pumped into and out of the lungs being 13.6 litres per min., with 180 strokes per min. and 75 c.c. per stroke.

The animals used in all the experiments in Tables II A and B were healthy and on normal diet. An experiment was carried out on a cat (2.2 kg.) which had been starved for 48 hours. Before artificial respiration the  $\text{CO}_2$  and  $\text{O}_2$  c.c. per hour were 864, the R.Q. being 1. After only 24 mins. artificial respiration, pumping at the rate of 7.3 litres per min., the  $\text{CO}_2$  and  $\text{O}_2$  had fallen to 570 c.c. per hour, indicating that starvation prevented the metabolism from rising. It seemed probable then that the metabolism was increased in the healthy animal at the expense of easily mobilised reserve, e.g. glycogen of the muscles and liver.

*Effect of artificial respiration on the glycogen content of the liver.* Four experiments were carried out to determine whether artificial respiration would definitely reduce the glycogen content of the liver, using Pflüger's method of estimation (Table III). In all the experiments the animals were fed on carrots, two meals being taken per day. Care was taken that the control animals were treated in exactly the same way as the experimental animals, with the only difference that the experimental animals were subjected to artificial respiration whereas the controls were not.

In Exps. 1 and 2 the glycogen content of the liver was estimated at the end of the experiment only. From such results alone one is not entitled to draw conclusions because, whatever precautions were taken, the glycogen content of the livers of the two animals might not have been similar at the start of the experiment. In order to attempt to exclude such an error in Exps. 3 and 4, a portion (about 6 g.) of the left lobe

of the liver was tied off by tape and removed in both the control and the experimental animal before performing the artificial respiration; whilst a portion of the right lobe of each animal was removed after cessation of the artificial respiration in the experimental animal. The difference between the glycogen content at the beginning and at the end of the experiment in the control animal gave the effects of the anæsthetic and the operations; whilst in the experimental animal, the difference between the glycogen content at the beginning and at the end of the experiment gave the effects of the anæsthetic and operations *plus* the effect of artificial respiration. Thus in Exp. 3 the left lobe of liver of the control animal contained 16 mg. of glycogen per grm. of gland at the beginning and the right lobe of the liver 14 mg. at the end of the experiment. Whereas in the animal subjected to artificial respiration for 20 mins. the left and right lobes of the liver contained 15 mg. and 3 mg. respectively, showing that artificial respiration had greatly reduced the glycogen content of the liver. The same effect is shown in a much longer Exp. 4; the left lobe of the liver of the control animal contained 26 mg. at the beginning of the experiment and the right lobe 17 mg. of glycogen per grm. of gland at the end, whereas the left lobe of the liver of the animal subjected to artificial respiration for 84 mins. contained 18 mg. before and the right lobe only a trace of glycogen after the 84 mins. of pumping. In these few experiments it seems evident that glycogen of the liver was used to counteract the effects of the pumping out of  $\text{CO}_2$ , the glycogen probably being carried, as sugar, in the blood to the tissues to be metabolised to replace  $\text{CO}_2$  pumped out. There still remains a possible source of error in the above method in that the left and right lobes of the liver might have possessed different glycogen contents at the start of the experiment. Because the effects of artificial respiration appeared to be so marked and because the figures for the left and right lobes of the controls showed not nearly so great a difference, this source of error can reasonably be excluded.

The animals in Exps. 3 and 4 were killed when the experimental animal showed signs of dying as estimated by the corneal reflex, that is, artificial respiration was carried out until this reflex had become very weak.

*The effects of intravenous injections of alkali and acid on metabolism of anæsthetised cats.* Dale and Evans(3) showed that during the performance of vigorous artificial respiration, the blood, both arterial and venous, became alkaline. It was thought that this alkalosis might be partly responsible for the increased output of  $\text{CO}_2$  and increased intake of  $\text{O}_2$  in the present research. Several experiments were therefore carried out

in which 10 p.c.  $\text{NaHCO}_3$  was injected into a vein of a cat under urethane and ether anaesthesia; a moderate corneal reflex was obtained, during the time the metabolic rate was estimated. The metabolism was estimated by the modification of the Douglas-Haldane method already mentioned.

Table IV gives the results obtained; the  $\text{CO}_2$  production and the  $\text{O}_2$  consumption were constantly and definitely increased after the injection of  $\text{NaHCO}_3$ ; whereas the volume of air breathed varied considerably and apnoea was never observed. The average increase in  $\text{O}_2$  intake was 16 p.c.; the samples of air collected covered 10 to 20 mins. Warburg(4) showed that addition of  $10^{-4}$  normal sodium hydroxide to sea-water doubled the  $\text{O}_2$  consumption of developing sea-urchin eggs. Macleod and Knapp(5) found that as a result of alkalinity produced by injection of sodium carbonate into the blood stream, the lactic acid production in the tissues was stimulated. It was noted in the present experiments that injection of  $\text{NaHCO}_3$  produced vomiting<sup>1</sup> which soon passed off, and sometimes passage of urine. Metabolic rate was not estimated until about 20 mins. after the injection or 20 mins. after the cessation of vomiting.

Table IV also shows the effect of intravenous injection of  $\text{HCl}$  upon  $\text{O}_2$  consumption. There was a slight but constant decrease of  $\text{O}_2$  intake in this case, an effect opposite to that obtained with  $\text{NaHCO}_3$ . Attention is directed to the figures for volume of air expired. There was no marked hyperpnoea in these observations, probably because, as others have already pointed out, the anaesthesia had rendered the respiratory centre insensitive to changes in blood reaction. The samples of air collected were taken about 20 mins. after an injection and covered from 10 to 20 mins. A moderate corneal reflex was elicited during the taking of the samples. Barr(6) has recently observed that following normal exercise in man the respiratory centre was not so sensitive to changes in reaction of the blood as has been supposed. My observations, at any rate, indicate that in the absence of such sensitivity of the respiratory centre the metabolism tended to be depressed by  $\text{HCl}$ .

Table V gives the details of results of an experiment wherein both acid and alkali were injected into the same animal. It is seen that acid reduced the  $\text{O}_2$  consumption markedly after it had been increased by alkali. Care was taken to clear the cannula and the vein of  $\text{NaHCO}_3$  before injection of  $\text{HCl}$ , in order to prevent large formation of free  $\text{CO}_2$ . The average difference between  $\text{O}_2$  consumption after the  $\text{NaHCO}_3$  and after  $\text{HCl}$  was 24 p.c. in my experiments; that is, it was raised 16 p.c.

<sup>1</sup> King and Church (*Am. J. Physiol.* 62, p. 459, 1922) have found that intravenous injection of  $\text{NaHCO}_3$  calls forth a vigorous motor reaction of the small intestine of the dog.

after injection of the alkali and lowered 8 p.c. after the injection of the acid. When the respiratory centre was apparently insensitive to alteration in H-ion concentration, the body metabolism attempted to correct the condition. Increased  $O_2$  intake during increased alkalinity and decreased  $O_2$  intake during increased acidity probably constitute a general physiological principle. The reason why previous observers have not noticed these phenomena may be that the effects on metabolism were masked by other effects, *e.g.* on the respiratory centre.

The quantities of  $NaHCO_3$  and of  $HCl$  injected in my experiments were not too great for vital processes, since similar quantities were injected into the ear veins of normal unanæsthetised rabbits, with only temporary discomfort. Care was taken to inject the solutions very slowly. After injection of 10 c.c. 10 p.c.  $NaHCO_3$  into an ear vein of a rabbit the alveolar  $CO_2$  tension and the  $CO_2$  tension in air under the skin were markedly increased from 41 mm. to 54 mm., a result of some significance in the present discussion. The counterpart of this experiment, in which the alveolar  $CO_2$  tension and the  $CO_2$  tension in air under the skin were decreased by  $HCl$  was described in the previous paper(1). Previous observers obtained similar results in normal man. Davies, Haldane and Kennaway(7) showed that experimental alkalosis increased the alveolar  $CO_2$  tension in man and Haldane(8) demonstrated that experimental acidosis decreased the alveolar  $CO_2$  tension in man.

From the experiments described herein it seems reasonable to conclude that acid tends to decrease the  $O_2$  consumption and the production of  $CO_2$  throughout the body whilst alkali tends to increase these, independently of the respiratory centre.

#### SUMMARY.

1. Artificial respiration decreases the  $CO_2$  tension throughout the body, the lowest figures obtained being 21 mm. under the skin, 12 in the abdominal cavity and 7.5 in the arterial blood.

2. Artificial respiration increases the  $O_2$  consumption, the body cells attempting to replace the  $CO_2$  pumped out.

3. Artificial respiration decreases the glycogen content of the liver.

4.  $NaHCO_3$  injected intravenously increases, whereas  $HCl$  decreases the  $O_2$  consumption and  $CO_2$  production, independently of the respiratory centre.

I am indebted to Dr Leonard Hill and to Dr H. H. Dale for granting every facility in the above research. My thanks are also due to Mr T. A. Webster for assistance with the Van Slyke and glycogen estimations.

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TABLE I (average figures) Cats and rabbits, urethane and ether, artificial respiration (A R)

No of obs	Kilos	A R		CO <sub>2</sub> mm in air under skin	
		Duration mins	Litres per min	Before A R	After A R
3	2.7	139	8.9	41	30
5	2.6	92	11.1	CO <sub>2</sub> mm in air in abd cav	
				Before A R	After A R
				45	29

TABLE II A (average figures) Cats, urethane and ether, metabolism before artificial respiration

No of obs	Kilos	CO <sub>2</sub> c.c per kilo and per hr	O <sub>2</sub> c.c per kilo and per hr	Expired air				Blood pressure mm Hg
				Vol litres per min	CO <sub>2</sub> %	O <sub>2</sub> %	R Q	
5	2.5	254	324	0.34	3.44	4.47	7.69	102

TABLE II B Cats, urethane and ether, metabolism during artificial respiration (A R)

Kilos	Length of period of A R mins	CO <sub>2</sub> c.c per hr	O <sub>2</sub> c.c per hr	Air pumped out from lungs				Blood pressure carotid art		Result of A R
				Volume litres per min	CO <sub>2</sub> p.c	O <sub>2</sub> p.c	R Q	During A.R	After A R	
2.5	60	1200	1200	4.8	42	42	1.000	—	—	—
"	98	1560	1200	9.3	28	20	1.400	—	—	died
3.9	17	3240	4260	13.3	41	50	802	47	80	recovered
"	41	3900	6000	15.5	41	63	651	42	80	"
3.0	76	1710	2448	13.0	21	30	700	50	92	"
2.4	65	918	1414	11.7	13	20	650	28	47	died
1.9	5	1608	2160	8.4	32	43	744	43	73	recovered
"	15	1260	1656	7.3	29	38	763	33	93	"
"	30	1440	1440	7.9	33	33	1.000	43	43	died
3.0	104	897	1259	11.5	13	19	684	35	35	— <sup>1</sup>
2.4	27	1938	2412	13.4	24	30	800	35	35	— <sup>1</sup>
2.7	—	1788	2314	10.6	29	35	829	—	—	—

Average CO<sub>2</sub> c.c per kilo and per hr = 663Average O<sub>2</sub> c.c per kilo and per hr = 857

These animals were killed by taking samples of blood from arteries and heart during A.R.



urethane (one gram per kilo. of body weight injected subcutaneously). The respiratory movements were recorded by a method previously described by Mellanby (8) which ensures that the inspired and expired gases do not mix, and that therefore the respiratory movements are not affected by an accumulation of carbon dioxide or a deficiency of oxygen in the inspired air. The adrenalin was injected into the central end of a jugular vein, the usual dose being 1 c.c. of a .01 p.c. solution in Ringer's fluid.

*Afferent impulses from the viscera.* Kahn suggested that the two important areas from which visceral impulses might arise and cause adrenalin apnoea were the abdomen and the thorax.

(a) Roberts showed that stimulation of the central end of a cut splanchnic nerve never caused apnoea, and that the injection of adrenalin into the aorta below a ligature placed above the coeliac axis produced practically no effect on respiration. In confirmation of these results we find that injection of adrenalin into a jugular vein produces apnoea after section of both splanchnic nerves.

(b) Both vagus nerves were cut in the neck. Respiration assumed the usual slow and deep type. The intravenous injection of adrenalin produced characteristic apnoea. This result showed that afferent impulses from the thorax are probably not responsible for adrenalin apnoea. Against this conclusion is the well-known fact that stimulation of a certain strength of the central end of the vagus invariably causes cessation of respiration. We discuss this point in a subsequent section of this paper.

(c) The confirmatory proof that afferent impulses from the viscera or the general tissues of the body do not cause adrenalin apnoea was obtained from experiments in which the spinal cord was cut at different levels. Thus, in one experiment the spinal cord of a rabbit was severed at the level of the 2nd dorsal nerve. The blood fell to 37 mm. of Hg but

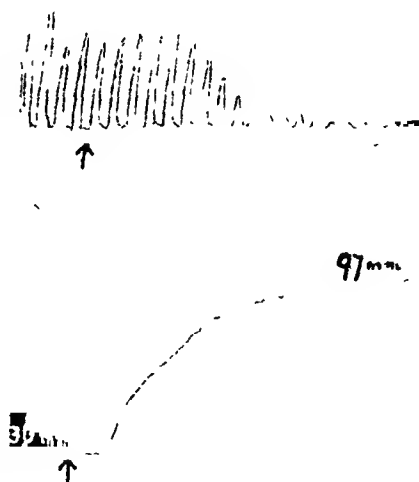


Fig. 1. Rabbit. Urethane. Cord cut at 2nd dorsal nerve. Effect of adrenalin (1 c.c., .01 p.c.) on respiration, and on the arterial blood-pressure.

The blood fell to 37 mm. of Hg but

the respiratory movements were practically unaffected. On intravenous injection of adrenalin the arterial pressure rose to 97 mm. of Hg and typical apnœa was produced (Fig. 1). The result showed that afferent impulses from parts of the body innervated below the 2nd dorsal nerve are not concerned in the production of adrenalin apnœa. The blood-pressure effects confirmed an observation of Boruttau that adrenalin apnœa is produced as readily in an animal with a low as with a high blood-pressure.

*Changes in the cranium.* We investigated the possibility that adrenalin apnœa is due to: (a) increased intracranial pressure; (b) impulses coming down to the respiratory centre from the fore-brain. For (a) the temporal bone of a cat was trephined, and the contents of the cranium exposed to the atmospheric pressure. The subsequent injection of adrenalin produced typical apnœa. For (b) a cat was decerebrated by Sherrington's decerebrator. Typical apnœa was produced by adrenalin (Fig. 2). From these

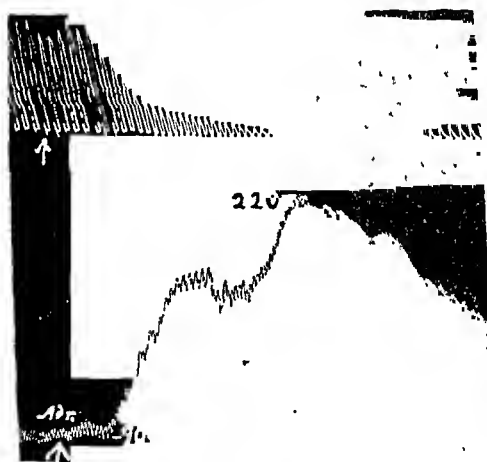


Fig. 2. Cat. Urethane. Decerebrated. Effect of adrenalin (1 c.c., .01 p.c.) on respiration and blood pressure.

results it is evident that adrenalin apnœa is not produced secondarily to pressure changes in the cranium or impulses from the fore-brain.

*The influence of ergotoxin.* From the results described it was necessary

lung ventilation can be obtained under normal conditions after section of both vagi. Under these circumstances, therefore, on Hering and Breuer's hypothesis the centripetal paths for inspiratory and expiratory impulses to the respiratory centre must arise in the inspiratory and expiratory muscles and travel in their afferent nerves to the spinal cord. Corresponding with this conception of the automatic regulation of respiration, a number of observers have assigned a purely inhibitory function to the afferent fibres of the vagus from the lungs. Thus, Gad<sup>(14)</sup> found that chemical stimulation of the vagus produced inhibitory effects only, whilst Patrizi and Franchini<sup>(15)</sup> observed that central stimulation of the vagus produced inhibition of respiration. Scott<sup>(16)</sup> re-investigated the functions of the afferent pulmonary fibres of the vagus in the light of Haldane and Priestley's<sup>(17)</sup> work on the chemical control of respiratory movements. He concluded that the vagus functions as the afferent nerve in the respiratory reflex, in a manner similar to other afferent nerves in reflex actions. His figures indicate that central vagus stimulation produced inhibition of inspiratory movements only. In the experiments recorded in this paper the invariable effect of moderate electrical stimulation of the central end of the vagus in rabbits and cats under urethane anaesthesia was arrest of respiratory movements in the expiratory phase. There was no evidence during the cessation of respiration of either inspiratory or expiratory tetanus—in fact, the arrest of respiration was a true apnoea in that during its occurrence the respiratory centre ceased to function. This vagal apnoea was similar to adrenalin apnoea in many respects and led us to test the assumption that aberrant sympathetic fibres travelled to the blood vessels of the bulb by the vagus nerves.

In his investigation Scott brought out the striking fact that stimulation of the vagus nerves causes apnoea even at the height of dyspnoea. We find that precisely similar facts hold for adrenalin. The intravenous injection of adrenalin during the dyspnoea produced by oxygen want (caused by the injection of sufficient sodium hydrosulphite,  $\text{Na}_2\text{S}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ , into the blood to reduce the oxyhæmoglobin) immediately annuls the exaggerated respiratory movements and causes complete apnoea. Similar facts hold for the dyspnoea produced by breathing air containing an excessive quantity of  $\text{CO}_2$ . Fig. 4 shows how carbon dioxide dyspnoea is immediately abolished by the injection of adrenalin. The tracing shows that when the adrenalin apnoea passes off, the exaggerated respiratory movements do not return. The adrenalin effect lasts so long that additional mechanisms in the body come into action to neutralise the

increased quantity of hydrogen ions circulating in the blood produced by the inspired carbon dioxide. The stimulation of the central end of a

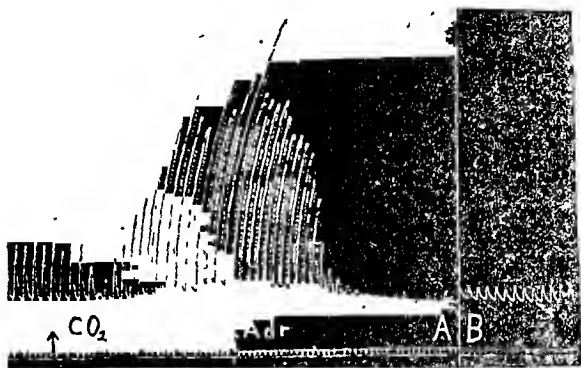


Fig. 4. Cat. Urethane. Effect of adrenalin (1 c.c., .1 p.c.) on carbon dioxide dyspnea. Interval of 2 mins. between A and B.

cut vagus during the dyspnea produced by oxygen want or by excess of carbon dioxide, causes apnœa similar in every respect to that produced by the intravenous injection of adrenalin except that the apnœa lasts for the duration of the stimulus only. This parallelism between adrenalin apnœa and vagus apnœa was also found in the decerebrate animal and in an animal with its cord cut in the upper dorsal region.

The above observations indicated that the vagus nerve carried aberrant constrictor fibres to the blood vessels of the bulb and that the cause of the vagal apnœa was essentially the same as that of adrenalin apnœa,

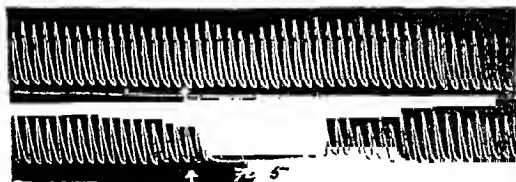


Fig. 5 Cat. Urethane. Effects of adrenalin (1 c.c., .01 p.c.) (upper tracing) and central vagus stimulation (lower tracing) on respiration after ergotoxin.

*i.e.* constriction of the blood vessels of the respiratory centre. The crucial experiment, however, did not support this idea; Fig. 5 shows that the intravenous injection of ergotoxin which abolishes adrenalin apnoea did not have any effect on vagal apnoea. Therefore the course of the constrictor fibres to the blood vessels of the respiratory centre is not *via* the vagus nerves.

The failure to find a definite nerve carrying sympathetic fibres to the vessels of the bulb suggests that the adrenalin acts on the cells of the respiratory centre as suggested by Boruttau. Such an action, however, would appear to demand that ergotoxin, which annuls adrenalin apnoea, should affect the sensitivity of the respiratory centre to other agents. In point of fact accurate experiments have shown that the response of the respiratory centre to afferent nerve stimulation or to changes in the quality of the blood (increased carbon dioxide or diminished oxygen) is unaffected by the intravenous injection of quantities of ergotoxin which completely abolish adrenalin apnoea. Probably, therefore, adrenalin produces apnoea by constricting the blood vessels of the respiratory centre independently of any sympathetic nerve supply to these vessels. On this assumption adrenalin apnoea would form another exception against the hypothesis that adrenalin stimulates sympathetic nerve endings only. This action would correspond with such exceptional effects as the fall of blood-pressure produced by minute doses of adrenalin, or the contraction of the muscle of the intestine produced by large doses of adrenalin. Further, this view of the anomalous action of adrenalin would accord with the fact that adrenalin apnoea forms an outstanding exception against the generalisation that adrenalin calls up the defensive mechanisms of the body. The action of adrenalin on the cerebral blood vessels as a whole is hardly relevant to this question which deals with localised areas and localised vaso-motor action. But it may be observed that Biedl and Reiner(18), Wiggers(19) and Wieschowski(20) found evidence of vaso-constriction on perfusing dilute adrenalin solutions through the blood vessels of the isolated brain.

#### DISCUSSION OF RESULTS.

The results indicate that the blood vessels to the respiratory centre are contracted by adrenalin. Since the contraction results in a diminution of respiratory movements, it is evident that lung ventilation is modified by the quantity of blood supplied to the respiratory centre. On this hypothesis the activity of the respiratory centre is determined not only by the quality of the blood supplied to it but also by the quantity of

blood supplied in unit time. This dependence of lung ventilation on both the quality and the quantity of the blood supplied to the respiratory centre may explain certain results which appear to contradict the hydrogen ion hypothesis of ventilation of the lung. Thus, in a series of experiments by Mellanby<sup>(21)</sup> it was observed that the respiratory movements of an animal breathing nitrogen or after the intravenous injection of lactic acid bore no relation to the reaction of the blood. Also, observations by Fraser, Ross and Dreyer<sup>(22)</sup> on the reaction of the blood in relation to dyspnœa in human beings, showed that dyspnœa may be associated not only with blood approximating to neutrality but also with markedly alkaline blood, and that blood much more acidie than normal may be present without dyspnœa. Probably in all these cases the quantity of blood supplied to the bulb in conjunction with the concentration of hydrogen ions in the blood determined the degree of lung ventilation.

The facts of adrenalin apnœa offer a proof that deficiency of oxygen does not lead to the formation of lactic acid in the cells of the respiratory centre and consequent dyspnœa. A diminution of the blood supply to the respiratory centre by adrenalin diminishes from the outset the amplitude of the respiratory movements. A momentary cessation of respiration might be due to the removal of an effective stimulus from the blood, *i.e.* the concentration of hydrogen ions in it. But on the lactic acid hypothesis the concomitant absence of oxygen should immediately lead to the intracellular production of lactic acid and thereby augment respiratory movements to a degree depending on the amount and duration of bulbar vaso-constriction. In this connection we have also found that adrenalin apnœa is not diminished in an animal whose blood and tissues have been saturated with oxygen by breathing pure oxygen for five minutes. It appears probable, therefore, that the hyperpnœa produced by oxygen want is not due to the formation of lactic acid in the cells of the respiratory centre.

Finally, it may be observed that the facts of adrenalin apnœa indicate that the respiratory centre deprived of blood although in connection with all afferent nerves possessing no automatic rhythmicity, in contrast to the observation of Rosenthal<sup>(23)</sup> that the respiratory centre freed from the influence of all afferent nerves but supplied with blood still discharges rhythmic impulses.

The marked similarity between vagal apnœa and adrenalin apnœa allows a comparison to be made between inhibitory phenomena obtained in two different ways, *i.e.* by vascular changes and by afferent nervous impulses. We may assume that stimulation of the respiratory centre is

caused by the hydrogen ions in the blood. Under the influence of adrenalin the respiratory centre is deprived of blood and hence this stimulus ceases to act; in the case of vagal apnœa probably the afferent nervous impulse so affects the membranes of the cells that they become impermeable to the hydrogen ions of the blood.

#### SUMMARY.

(1) The only method by which adrenalin apnœa can be prevented is by the intravenous injection of ergotoxin.

(2) Adrenalin apnœa is caused by the constriction of the blood vessels supplying the respiratory centre. No sympathetic nerve supplying the blood vessels has been found. Probably adrenalin constricts the blood vessels of the respiratory centre independently of any sympathetic innervation.

(3) Vagal apnœa is comparable to adrenalin apnœa, except that it is not annulled by ergotoxin.

(4) It is suggested that in vagal apnœa afferent impulses so affect the cells of the respiratory centre that their membranes are no longer permeable to the hydrogen ions in the blood which normally initiate respiratory impulses.

We wish to place on record our thanks to Prof. J. N. Langley for his criticisms and suggestions.

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# THE ACTION OF VASO-CONSTRICTOR SUBSTANCES ON THE ARTERIES OF THE BRAIN.

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IN a previous communication<sup>(1)</sup> I have given reasons for believing that the apnœa which follows the intravenous injection of adrenalin in rabbits and cats is to be attributed to vaso-constriction in the respiratory centre. I have since found that Loewi and Meyer<sup>(2)</sup> had expressed the same view for more or less the same reasons. This view has received additional support from Mellanby and Huggett<sup>(3)</sup>, who confirmed my results and showed further that the arrest of respiration did not occur if ergotoxin had previously been given, this fact indicating that the paralytic action of adrenalin was due to vaso-constriction and not to direct inhibition of the centre. The further demonstration by myself<sup>(4)</sup> that Cheyne-Stokes respiration when produced by adrenalin is associated with periodic opening and closing of the cerebral vessels seems to me to put this explanation beyond any doubt. But to many minds such a view is very difficult to accept and this for two reasons. First, the post-mortem perfusion of the brain with adrenalin has yielded widely different results in different hands; secondly, if a vaso-constrictor action of adrenalin on the brain were admitted it would be strong though not conclusive evidence that the cerebral vessels are under sympathetic control and this is now denied by the majority of physiologists. If, therefore, it can be shown that vaso-constrictor substances for which there is no *a priori* reason for excluding an action on the cerebral vessels produce the same changes in respiration as have been shown to follow adrenalin we shall be confirmed in our view that adrenalin has a cerebral vaso-constrictor effect since the only alternative is the very improbable coincidence that all vaso-constrictor substances directly inhibit the respiratory centre. In these pages I present the results of experiments performed with this object, the substances whose action has been investigated being pituitary extract, ergotoxin and barium chloride.

*Pituitary extract.* A number of investigations have been made upon the respiratory effect of pituitary extract with very different results. Mummery and Symes<sup>(5)</sup> noted a diminution in the amplitude. Houghton and Merrill<sup>(6)</sup> observed in the dog an increase in frequency



but owing to the limitation of their method they were unable to record any change in depth. A very full account of the effect both upon circulation and respiration in the rabbit is given by Paukow(7). Paukow using the Parke-Davis preparation showed the striking fact that with vagi intact there were always two periods of apnoea separated by a period of respiration, the first or "primary" apnoea which occurred immediately after the injection while the "secondary" apnoea which occurred during the recovery of blood-pressure from the profound fall which usually occurs in this animal. After atropine or after section of the vagi the primary apnoea, he said, did not occur. Paukow attributed the primary apnoea to a reflex inhibition of the centre set up by stimulation of afferent nerve-endings. The secondary apnoea he was unable to explain but suggested that it might be due to contraction of the bronchioles. Paukow further noticed that after the secondary apnoea the respiratory tracing often became spindle-shaped but he seems not to have realised that he was dealing with Cheyne-Stokes respiration. He thought that this effect might be partly the result of the anæsthetic. Fühner(8) approaching the subject from a pharmacological standpoint came to the same general conclusions but considered that the secondary apnoea only was characteristic of the specific principle of pituitrin, the primary apnoea being due to choline. In 1913 Fröhlich and Pick(9) studied the effect of various pituitary preparations upon tracheotomised but non-anæsthetised rabbits. They measured the volume of expired air and found considerable diminution accompanied by powerful expiratory efforts. This corresponded in point of time to Paukow's primary apnoea. It was abolished by atropine but, contrary to Paukow's result, not by section of both vagi. They believed that the effect was due to bronchial constriction. Paukow's secondary apnoea they attributed, following Loewi and Meyer's explanation of adrenalin apnoea mentioned above, to anæmia of the medulla by vaso-constriction. They supported this view by showing that this apnoea did not occur when pituitrin was given mixed with amyl nitrite so as to counterbalance the pressor effect. Paukow's secondary apnoea they termed "indirect" apnoea. Nice, Rock and Courtright(10) using cats and dogs found in general an increase in depth followed by shallowness. It appears, however, that the dose which they gave (usually the extract diluted 10 or 25 times) was too small to produce the characteristic effect upon blood-pressure. Weed and Cushing(11) on injecting the "hypophysin" preparation of Lucius, Meister and Brunig found an increase in the depth of respiration. Dixon and Halliburton(12) also found increase of respiration but they attributed

it to dyspnœa caused by contraction of the bronchioles. As the bronchial contraction took 30 seconds to be complete the effect on respiration did not occur at once. The only experiments upon the excised cerebral arteries are those of Cow (13) who found no appreciable effect.

The following experiments were made upon rabbits and cats anæsthetised with urethane supplemented with a little C.E. mixture. The preparation used was the "pituitrin" of Messrs Parke-Davis and Co. It was given intravenously. Respiration was recorded as in previous experiments by connecting one limb of the Y-shaped trachea tube to a rubber tambour. In their work upon the bronchi Dixon and Halliburton have pointed out that this is not a good method of recording bronchial obstruction whether due to bronchial contraction or to vascular congestion such as they find is produced by adrenalin and other substances which increase systemic pressure. But since bronchial obstruction does not prevent respiration from affecting intra-tracheal pressure this method can be safely used to show whether there is apnœa or not. No doubt at the moment of obstruction the record does not accurately represent the change in strength of respiratory movements. I have, however, in all experiments watched the movements and found them to correspond with the tracings. Moreover, in one experiment (Fig. 1) I have taken a simultaneous tracing of the abdominal movements. The tracings are essentially the same.

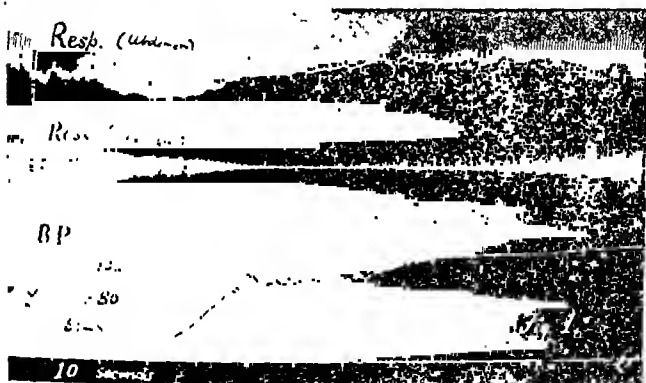
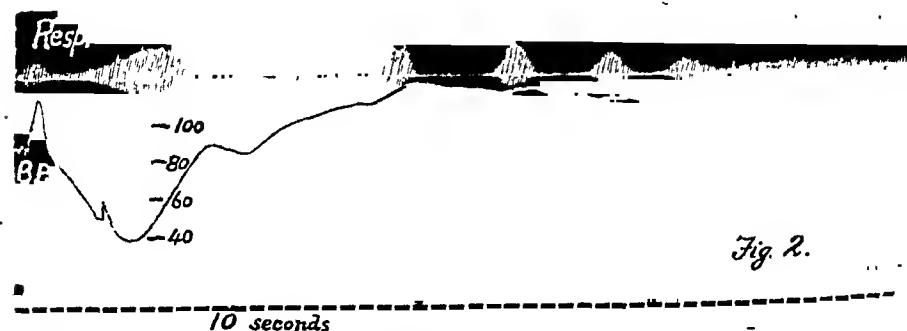


Fig. 1. Rabbit. Vagi intact. 1 c.c. pituitary intravenously. Typical effect.

We shall first deal with the rabbit. A typical effect of the injection of 1 c.c. is shown in Fig. 1. There is first an immediate diminution in the depth of respiration usually amounting to complete apnoea during which the chest is completely at rest. This lasts about 10 seconds and is usually accompanied by a transient rise of blood-pressure of variable degree. Rarely no rise occurs. Following this, the pressure undergoes a profound fall. During the fall respiration recommences progressively increasing in depth. Blood-pressure then slowly recovers, the tracing showing a much slower heart-beat. At the beginning of the rise respiration undergoes a progressive diminution in depth until a second apnoea sets in. This is more prolonged than the first apnoea and lasts sometimes until the blood-pressure reaches its summit which is often not much higher than its original level. Respiration is then gradually resumed. Besides these changes in depth there are usually changes in frequency. The respiration which follows the first apnoea is commonly at a much faster rate than the original but this alteration usually only occurs if the first apnoea is complete. It would appear that a new rhythm cannot be adopted unless the old rhythm is first completely suppressed. The respiratory rate following the second apnoea may be at the immediately previous rate or at the original rate or again at a totally new rate. Following the second apnoea Cheyne-Stokes respiration may occur, the extent to which it occurs depending as in the case of adrenalin upon individual susceptibility.

With vagi cut the appearances are in general the same. The first apnoea, however, tends to be less marked, a fact which may be associated with the slower rate of respiration. Further, the rate of respiration is usually unaltered throughout. An example is seen in Fig. 2 which shows in addition an unusual degree of periodic respiration.



*Fig. 2.*

Fig. 2. Rabbit. Vagi cut. 1 c.c. pituitary. Well-marked Cheyne-Stokes respiration. Apart from the apnoeic periods there is no alteration in rate of breathing.

It will be seen that the results are more difficult to interpret than those obtained with adrenalin. Owing to the complex manner in which the cardio-vascular system is involved it seems at first sight difficult to correlate changes in respiration with those in the arterioles. It may be stated here that the first and second apnoea occur irrespective of gross changes in general blood-pressure. Fig. 3 shows that they occur when the

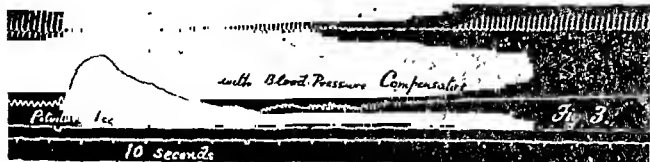


Fig. 3. Rabbit. 1 c.c. pituitary. Blood-pressure partially compensated.

general blood-pressure is largely counterbalanced by the use of the compensator previously described. At the same time it is important to know whether the great fall in pressure is cardiac or peripheral in origin. Paukow believed that it was cardiac. I have confirmed this by direct inspection of the heart. A large hole was made in the left side of the chest and the pericardium opened so that a good view of the heart could be obtained, the animal meanwhile breathing naturally with the right lung. It was easy to see that the fall in blood-pressure was accompanied by considerable embarrassment of the heart with enormous dilatation of the auricles. The blood-pressure therefore during this period gives no indication of the state of the arterioles.

As regards the Cheyne-Stokes respiration it is clear from Fig. 2 that there is an intimate relation between respiration and blood-pressure for the tracing shows quite clearly a fall in pressure preceding and during the development stage of each respiratory period and a rise in pressure during the stage of subsidence. The similarity in this respect between adrenalin and pituitrin is most striking. Unfortunately no record of cerebral pressure was taken in this experiment but the result, assuming that there is no variation in the action of the heart of which there is no evidence, shows that when the centre is reacting in this periodic manner it becomes active after and during vaso-dilatation and quiescent during vaso-constriction.

We have now to consider the first and second apnoeic periods. It will be convenient to take the latter first. The second apnoea commences

atropine, a dose sufficient to paralyse the vagal fibres to the heart, this apnœa still occurs. It seemed possible that the positive effect which I obtained might be due not to pituitrin but to the too rapid injection of fluid. This was easily put to the test by injecting rapidly into the jugular vein 1 c.c. of warm Ringer solution. Respiration was unchanged. The explanation of the negative results of Fröhlich and Pick is, I think, to be found in the very slow rate of injection—on one occasion 1 c.c. in 60 seconds.

The primary apnœa has nothing to do with the bronchial constriction found by Fröhlich and Pick in their unanæsthetised animals. In the first place it has been shown by Dixon (unpublished observations) that the constrictor effect of pituitrin on the bronchioles is completely abolished after urethane. In the second place the apnœa produced under the circumstances of my experiments was always a true apnœa, in which all the respiratory muscles were either completely at rest or very much suppressed. There was no trace of the dyspnœa which invariably indicates bronchial obstruction.

That the first apnœa is central in origin is shown by the fact that when pituitrin is injected directly into the cerebral vessels as in Fig. 4 the apnœa ensues *immediately*, that is, before the drug has time to get into the general circulation. That it is caused by cerebral vaso-constriction is suggested by the fact that it usually occurs contemporaneously with an initial rise of blood-pressure as in Fig. 1.

The action of pituitrin on the cat calls for little comment. The simple rise in blood-pressure is accompanied by diminution in the depth of respiration. This is undoubtedly the typical effect. In the experiments already quoted of Nice, Rock and Courtright upon the cat and dog

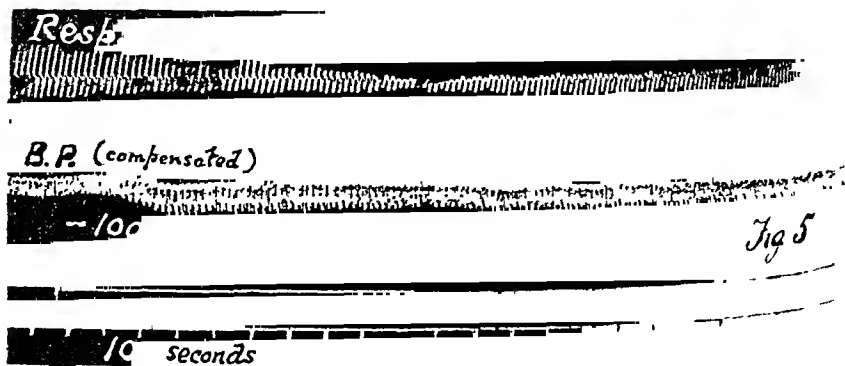


Fig. 5. Cat. Ergotoxin phosphate, 3 mgms. Blood-pressure compensated.

and in those of Weed and Cushing upon the dog the doses given were such that the typical rise of blood-pressure did not occur. This explains why these observers failed to obtain diminished respiration.

*Ergotoxin.* Fig. 5 shows the marked suppression of respiration which occurs in the cat when ergotoxin phosphate, 3 mgms., is given intravenously. (I am indebted to Dr Dixon for the ergotoxin used.) The respiratory effect was not due to the rise in pressure since this was completely neutralised by the use of the compensator.

*Barium.* Fig. 6 shows the complete respiratory pause following the

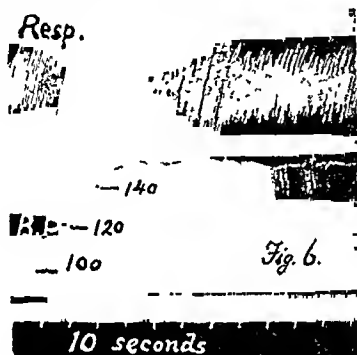


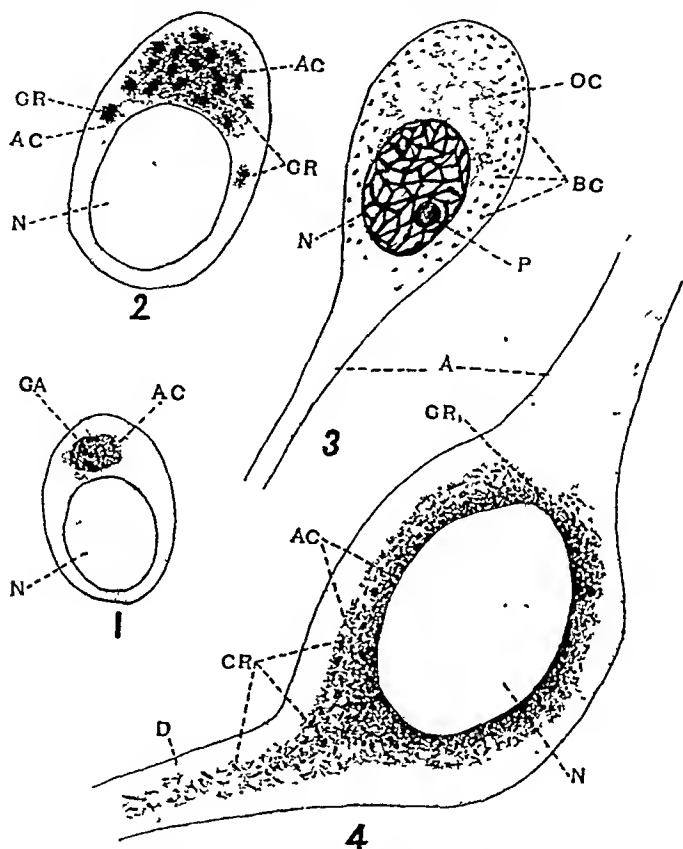
Fig. 6. Rabbit. Barium chloride, .01 gm.

injection of barium chloride, .01 gm. made up in Ringer solution. Barium is peculiar in that the respiratory effect is only of short duration whereas the raised blood-pressure continues for a very long time as is well known. This point requires further investigation. Two tentative explanations suggest themselves. The first is that the cerebral vaso-constriction passes off sooner than the vaso-constriction in the rest of the body. The second is that the vaso-constriction is only of moderate degree so that the medulla resumes its function when the hydrogen ion concentration of the blood has, owing to the apnoea, increased so as to counterbalance the diminished amount of blood supplying the centre.

#### SUMMARY.

1. The three vaso-constrictor substances tested, namely pituitrin, ergotoxin and barium chloride, all cause a diminution or complete stoppage

In the larger neurones a zone of the cytoplasm immediately surrounding the centrally placed nucleus, and in which the Golgi rods lie, appears darker than the remainder. This heavily impregnated cytoplasm



Figs. 1-4. Neurones of *Helix aspersa*

A = Axon. AC = Argentophil cloud. BG = Basophil granules (tigroid body?). D = Dendron. GA = Golgi apparatus in extra-centric position. GR = Golgi rods scattered. GR<sub>1</sub> = Small Golgi rods in region of axon-hillock. N = Nucleus. OC = Oxyphil cloud. P = Plasmosome. Fig. 1. Small neurone with the Golgi apparatus, surrounded by an argentophil zone, in the perinuclear extra-centric position. Da Fano preparation.

Fig. 2. Medium sized neurone with Golgi rods scattered at one end of the nucleus. Rods surrounded by argentophil cloud which is darker in immediate neighbourhood of each Da Fano preparation.

Fig. 3. Medium sized neurone, cut in plane of axon, showing oxyphil cloud in similar position to argentophil cloud in 2. Basophil granules present in peripheral regions. Hæmatoxylin-eosin preparation.

Fig. 4. Large neurone with Golgi rods in the argentophil cloud around the nucleus and extending into dendron. These are absent from the axon and not well marked at its base. Da Fano preparation.

does not appear granular but is as homogeneous as in other parts of the cell. The Golgi rods at the base of the axon-hillock appear to be fewer and smaller than on other sides of the nucleus and here also the dark zones are less well marked and narrower than in other places. No similar alteration of either the Golgi rods or zones occurs in relation to the bases of the dendrons. In some cases the zones are darker near the nuclear membrane than on the outside, but in all they correspond with the distribution of the Golgi apparatus (Figs. 7 and 8).

In the medium sized neurones the nucleus is often excentrically placed, the Golgi rods being scattered around one end, probably that farthest from the axon (Fig. 6). Where this arrangement occurs the perinuclear zone of the larger neurones is replaced by an excentric cloud, in which the Golgi rods lie, capping one end of the nucleus. In many cases this cloud is easily observed to be much darker around each individual rod.

In small neurones in which the Golgi apparatus is in the extra-eentric perinuclear position, or is breaking up to assume the diffuse arrangement, each individual rod or group of rods is surrounded by a separate dark zone of its own (Fig. 5). I have therefore no hesitation in submitting that a causal relation exists between the Golgi rods (and their archoplasm) and these zones.

*Changes of chromophility in the neurones of Helix.* In the light of the above results, and of some recent work by Gatenby(6) and by Hirschler(10) it seemed interesting to study the chromophility of the cytoplasm of these cells. For this purpose Scott's technique, in a slightly modified form, was employed. Sections, of material fixed in Petrunkevitch's fluid, after being brought to water, were treated on the slide with iodine alcohol for three minutes. The iodine was removed by washing with 90 p.c. alcohol followed by .25 p.c. solution of  $\text{Na}_2\text{S}_2\text{O}_3$  in 50 p.c. alcohol. The  $\text{Na}_2\text{S}_2\text{O}_3$  was removed by washing in aq. dist. and then treated with 90 p.c. alcohol. After wiping the slides dry around the sections Ehrlich's hæmatoxylin was applied for ten minutes. The stain was removed by 90 p.c. alcohol followed by aq. dist. Lithium carbonate water was used to turn the hæmatoxylin blue and was subsequently removed by washing in aq. dist. Weak watery eosin was then applied and allowed to remain on the sections for a few minutes, after which they were quickly dehydrated in alcohols, cleared in xylol, and mounted in balsam.

Sections prepared by this method were well stained. In them, as well as could be seen, the cytoplasm of the small neurones was basophil.



In larger neurones an amphophil cloud was present surrounding the nucleus on all sides except that nearest the axon-hillock and was thickest



Figs. 5 to 8 are microphotographs of *Da Fano* preparations of the cephalic ganglia of *Helix aspersa*. They were taken on Wratten panchromatic films without the use of light screens and are in no way touched up.

Fig. 5. Small neurones with Golgi apparatus in perinuclear extra-centric position or commencing to disperse. The argentophil zone can be seen surrounding the apparatus.  $\times 1000$ .

Fig. 6. Two medium sized neurones. Golgi rods scattered in the cytoplasm at one end of the nucleus and surrounded by the argentophil cloud which is darker in the immediate vicinity of each rod.  $\times 1200$ .

Fig. 7. Large neurone, cut through the base of the axon-hillock, in centre. The Golgi rods surround the nucleus but are few and small at the end next the base of the axon-hillock (left in this figure). The argentophil cloud corresponds in distribution with the Golgi rods but is darkest near the nucleus. Neither extend into the axon.  $\times 200$ .

Fig. 8. Sector of same cell as shown in Fig. 7 at higher magnification. A darkening of the argentophil zone can be seen around each rod. It will be noticed that the darkening of the zone near the nuclear membrane corresponds with an increase in the number of rods in this region.  $\times 1000$ .

at the end farthest from it. In many cells this cloud had a distinct oxyphil preponderance. In cells of this size, small flocculent basophil

granulations were observable in the cytoplasm, especially near the periphery. In the large cells these granulations were more distinct and the cytoplasm, as a whole, was basophil, or amphophil with a strong basophil preponderance.

In all the cells the cytoplasm of the axon was oxyphil, but did not appear as dense as that of the cell body. I am inclined to think that this is due to the absence of the basophil substance from the axon, and that the oxyphility is present in other parts of the cytoplasm but masked by this substance.

I believe that these basophil granules represent the tigroid body and therefore their absence from the axon would be expected. In sections prepared by the same method, but with the omission of the eosin staining, no perinuclear cloud was observed in any of the cells. These results show that in cells of a certain size a densely staining cloud appears surrounding the nucleus. This cloud seems to be formed by the increase of some oxyphil substance or substances in the cytoplasm. From the facts that these clouds correspond with the distribution of the Golgi rods, as seen in silver and osmic preparations, and that they appear in cells of the same size as those in which Da Fano's method reveals the darkest zones around the apparatus, I conclude that they are also the result of its activity.

*The oocytes of Saccocirrus.* Gatenby<sup>(6)</sup> describes and figures perinuclear clouds in the oocytes of *Saccocirrus* prepared by Da Fano's method. He kindly allowed me to examine his preparations, which lead me to believe, as he suggested, that these clouds are identical with those shown in the neurones of *Helix* by the same method. I do not deny that the nucleus has some effect upon the production of these zones, but consider them to be directly produced by the Golgi apparatus; by the argentophil substance or the archoplasm, or by both combined. Gatenby also finds changes in chromophility during oogenesis. He says: "The oogonial cytoplasm is oxyphil. During oogenesis at the period of the appearance of the perinuclear bodies (nucleoli) the cytoplasm becomes basophil, especially near the nucleus. This basophilicity persists in a perinuclear position for a considerable time and spreads out, but gradually the entire cytoplasm again becomes completely oxyphil." He believes that the material which stains basophil is possibly the same as that which forms the perinuclear cloud in the cohalt-formol-silver-nitrate preparations. It is worthy of note, however, that at this period the nucleolar deutoplasm is also being formed and that it is distinctly basophil.

*Discussion.* In every cell at some period of its life the Golgi apparatus is in the perinuclear extra-centric condition. It then consists of a number of curved rods lying on the surface of the more or less globular mass of archoplasm or centrosphere. In the larger and more specialized forms of cells such as the neurone or the oocyte, the apparatus does not remain throughout life in this position. It becomes scattered, first breaking into pieces which, dividing further, pass around the nucleus, until finally it consists of single rods, probably curved and with portions of the archoplasm or centrosphere in their concave sides, scattered around the nucleus and throughout most of the cytoplasm. Each individual rod with its archoplasm, seems to have the power of independent binary fission. This power accounts for the exceedingly large number present in some oocytes and in large cells of many categories. In some highly specialized cells, such as the vertebrate neurone, the apparatus undergoes a still further elaboration, resulting in the formation of a robust reticulum surrounding the nucleus. It was in this stage that Golgi described it, as the "apparato reticulare interno," for the first time. As the apparatus generally occupies the extra-centric position in the youngest and least differentiated cells and in early stages of embryogeny I believe that it is permissible to assume that this is the most primitive condition. It is, however, very remarkable that even in the phylum Protozoa it has been shown<sup>(11)</sup> to become scattered at certain stages. It is also of much interest that it has been shown to remain a constant cell factor throughout gametogenesis and embryogeny. These facts, coupled with its behaviour during mitosis, afford a strong proof that it is a definite cell organ, a portion of the living protoplasm, and not merely the product of cytoplasmic or other activity. If it was a metabolic product it would be reasonable to expect it to reflect the changes in the activity of the cell, but no correspondence of this nature is observable.

The observations recorded above indicate that the Golgi apparatus gives rise to argentophil and oxyphil clouds in the cytoplasm. It is also worthy of note that the basophil granulations which I believe represent the tigroid bodies, are most marked in those cells in which the apparatus is very active. Perhaps they are produced by the activity of the apparatus. This theory is supported by the facts that in the vertebrate neurone the Nissl substance has the same distribution as the Golgi apparatus; neither are found in the axon and axon-hillock or in peripheral regions of the cytoplasm, and both extend into the dendrons. There are some previous observations which point in the same direction. Those of Gatenby have already been mentioned. Hirschler<sup>(10)</sup> has

described changes in the staining reactions of Ascidian oocytes corresponding with changes in the condition of the Golgi apparatus. He concludes that the apparatus serves to convey certain nuclear substances to the ground-plasm and suggests that it may, in fact, be concerned with its basophil reaction. Perhaps the basophil substances described by Gatenby and Hirschler are the same as the basophil granules (Nissl granules) I have described. If so the oxyphil cloud in the neurones of *Helix* is possibly another and separate product of the activity of the apparatus.

Possibly the so called chromatin emissions of Schaxel(13) represent a metabolic activity of the Golgi apparatus such as I have described.

I conclude that the function of the Golgi apparatus is connected with the cytoplasmic metabolism, possibly the production of protein substances. This result is in harmony with the views that the apparatus is concerned in secretion and in yolk formation. It seems to me also to follow on general grounds. Since it is a definite organ and has been found in all cells adequately investigated it must have one very general function. I see no other function which could reasonably be attributed to it except that of metabolism.

My thanks are due to Professor Gatenby for his advice in connection with the production of this paper and for his kindness in allowing me to use his preparations of *Saccocirrus*.

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## THE FUNCTION OF THE TUBULES IN KIDNEY EXCRETION. BY E. B. MAYRS.

*(From the Department of Pharmacology, Edinburgh.)*

It is becoming generally recognised that filtration through the glomeruli and some degree of re-absorption in the tubules are essential features of renal activity. The questions at issue are, in the first place, whether the kidney secretes at all; and secondly, whether the glomerular membrane is a simple ultra-filter in the ordinary physical sense, or can, by some vital process, retain those diffusible substances which are of value to the organism. Evidence of the constant presence of glucose in the glomerular fluid is now fairly strong; and since this is not a waste product but a substance of high physiological importance, the cells of Bowman's membrane have apparently no power of vital selection, but allow all the crystalloids of the plasma to pass through or between them without any change in concentration. It is intended, therefore, to deal in this paper with the question of secretion by the tubule cells.

Interesting observations have recently been published by Marshall and Vickers<sup>(1)</sup> and also by de Haan<sup>(2)</sup>, which seem to show that certain dyes are secreted by the cells of the convoluted tubules. It should be mentioned, however, that de Haan does not invoke secretion to interpret his results, but prefers the unlikely theory that proteins can pass through the glomerular membrane. This hypothesis does not explain certain results obtained by Marshall and Vickers. Neither is it in accord with the fact that gum arabic, if present in the blood in sufficient concentration, can produce almost complete anuria; for gum should pass through a membrane which allows the passage of protein.

Accumulation of dye in the cells of the kidney was observed many years ago by Heidenhain<sup>(3)</sup>, who regarded this as a stage in the process of secretion and concluded that urea is concentrated in a similar way. Cushny<sup>(4)</sup> has shown, however, that the amount of urea in the kidney is not sufficient to admit of the theory of storage, especially when allowance is made for the urea content of the fluid in the tubules. He has examined the excretion of glucose also, with similar results. The recent evidence of secretion of dyes is more conclusive than that of Heidenhain, and justifies further search for analogies to normal excretion. A

series of experiments has therefore been carried out in order to decide whether, if this power of secretion exists, the kidney can apply it in dealing with ordinary constituents of the plasma. The experiments bear a general resemblance to those of Cushny, but special efforts have been made to reduce as far as possible the extra-cellular portions of the substances estimated in the kidney. The observations were also more quantitative in character, since plasma concentrations were used for comparison with those of the kidney fluid. On the other hand, more operative interference with the kidney was required, and considerable diuresis was essential.

During the rapid excretion of urea and sulphate the kidney was quickly freed from blood and its total content of these substances determined, so that any storage which might occur in the renal cells could be observed. The error due to partially formed urine in the tubules was unavoidable, but was reduced to a minimum by diuresis, which prevented much concentration of this fluid.

*Method.* Rabbits were anaesthetised with urethane and eviscerated. A loose ligature was placed round the aorta above the left renal artery, and another was arranged to include the left ureter and both the left renal vessels close to the hilum of the kidney. A cannula was tied into the aorta below the renal arteries and was connected by rubber tubing to a large syringe. A capillary tube was introduced into the left ureter. A solution containing sodium sulphate and urea was then injected into the jugular vein, and soon after the injection was complete urine was collected from the left kidney for a period of 1 to 4 minutes. Blood was taken from the carotid artery, and at the same time the ligature on the aorta was tightened. Air was at once forced through the kidney from the syringe, and when bubbles appeared in the renal vein the ligature at the hilum was tied. The kidney was now dissected out, and, after removal of the vessels, ureter, and adherent fat, was weighed, transferred to a mortar, and ground to a homogeneous semi-fluid pulp. This pulp, or an aliquot part of it, was washed into an evaporating basin and dried at 90° to 100° C. It was again weighed and then thoroughly extracted by grinding with five or six changes of warm water, the total volume of which was known. Most of the suspended matter in the extract was removed by the centrifuge, and urea and sulphate were determined in suitable quantities of the fluid. Urea was estimated by hydrolysis with urease followed by vacuum distillation, and sulphate by the gravimetric method, the organic matter in the extract being first oxidised with potassium chlorate and hydrochloric acid. The urea and sulphate of the

plasma and urine were determined in the same way, except that before sulphate estimations in the plasma the proteins were destroyed by incineration.

The total fluid of the kidney is represented by the weight lost in drying. Part of this fluid was in the renal cells and part in the tubules; traces probably in the smaller blood vessels and in intercellular lymphatics. The urea and sulphate in the mixed fluid were calculated from the results, and compared with the concentrations found in the plasma and urine. The figures obtained in a number of experiments are given in the accompanying table.

No.	Wt. of rabbit, kilos	Inject'on. c.c.	Vol. of urine. c.c. per minute	% urea in plasma	% urea in kidney fluid	% urea in urine	% Na <sub>2</sub> SO <sub>4</sub> in plasma	% Na <sub>2</sub> SO <sub>4</sub> in kidney fluid	% Na <sub>2</sub> SO <sub>4</sub> in urine	
1	2.0	Urea 5 % Na <sub>2</sub> SO <sub>4</sub> 5 %	40.0	4.05	.380	.258	.605	.677	.504	1.085
2	2.0	" "	40.0	1.00	.272	.272	.717	.511	.452	1.466
3	2.2	Urea 10 % Na <sub>2</sub> SO <sub>4</sub> 10 %	33.0	1.15	.475	.412	.984	.752	.725	1.655
4	2.0	" "	20.5	1.00	.260	.181	.703	.489	.515	1.509
5	2.7	Urea 5 % Na <sub>2</sub> SO <sub>4</sub> 10 %	27.0	.53	.150	.206	.464	.504	.595	1.989
6	2.1	" "	21.0	.46	.201	.243	.520	.511	.641	1.922
7	2.5	" "	24.7	1.37	.177	.288	.365	.583	.299	1.418
8	2.5	" "	36.5	1.20	.308	.336	.427	1.052	1.046	1.562
Average ...			1.34	.278	.274	.598	.635	.597	1.576	

It is clear from these results that during excretion of urea and sulphate, under the experimental conditions described, their concentrations in the total fluid of the kidney do not differ much from their concentrations in the plasma. Where any material differences are observed, the records of exceptionally high and exceptionally low kidney concentrations are about equally divided. If the error of individual experiments is eliminated as far as possible by taking the average results of the series, the concentrations in the kidney and plasma are found to be practically the same. When it is remembered that the urea and sulphate in the partially formed urine of the renal tubules must have undergone some concentration, the small total content of these substances is even clearer evidence that there is no concentration in the renal cells. It may be objected that urea is perhaps shielded from the action of urease by forming a compound with the protein of secreting cells; but no similar explanation will cover the shortage of sulphate, since organic matter is oxidised before the estimation is begun.

The process of elimination of urea and sulphate is, therefore, entirely different from that which Marshall and Vickers describe in the secretion of dye; for these observers conclude that a certain amount of dye

is stored in the renal cells before concentration in the urine occurs. Urea and sulphate, on the contrary, do not appear to be stored in the renal cells, and their concentrations in these cells may even be lower than their concentrations in the plasma.

Two objections may be raised to the technique recorded above. In the first place, it may be argued that the interference with the kidney necessary for arranging ligatures on its vessels was so great as to prevent secretion from taking place. In some of Marshall and Vickers' experiments, however, in which part of the spinal cord had been destroyed, the kidney must have been in a much more unfavourable condition for secretory efforts; yet storage of dye in the renal cells was found to occur. Secondly, it may be objected that the concentrations of urea and sulphate in the plasma were already so high that storage in the renal cells at a greater concentration was impossible, owing to the large amount of work against osmotic tension which this would involve. But the concentrations of these substances were increased by some means during the formation of urine, and if the kidney can secrete them, part at least of this increase might have been expected to take place within the cells.

This introduces the question of whether true secretion of any constituent of the urine might be possible without intracellular concentration; for the process might consist simply in retention of water while the substance is passing through the free border of the cell. The secretion of hydrochloric acid by the stomach may be an example of some such process, alkali in this case being held back. In view of the toxicity of certain products which the kidney must excrete, the value of an arrangement of this kind is clear; although the advantage of concentration by absorption of water from the tubules is still more obvious. But in the experiments of Marshall and Vickers (the only evidence of secretion by the mammalian kidney on which any reliance can be placed) concentration of dye in the renal cells undoubtedly occurred; and the inference can be drawn that if the kidney could secrete the normal constituents of the urine, it would do so by a similar method. At present, therefore, concentration in the cells must be regarded as a necessary criterion of renal secretion.

A striking characteristic of the excretion of dyes, well shown in the experiments of de Haan, is the extent to which the concentration of free dye is raised in its passage through the kidney. The concentration may be 8000 times as high in the urine as in an ultrafiltrate from the plasma. If secretion ordinarily takes place in the kidney, some, at least,



of the constituents of the urine should be concentrated to a comparable degree. But even when the volume of urine is small, no increase of this magnitude is found to occur. Any doubt which exists in the case of creatinine, owing to the negligible amount in the plasma, can be removed by injecting this substance and observing its elimination; and the same method can be applied to ammonia also. In neither instance is the concentration very great. Hence, while it is not possible at present to dispute the secretion of dyes, it has yet to be shown that this process has any resemblance to the ordinary formation of urine.

In conclusion it is suggested that the primitive kidney may have had both secreting and absorbing functions, but that in the mammal a method of excretion has been selected which depends on filtration and re-absorption; only a few traces surviving of the earlier secretory power. The secretion of readily adsorbed substances, which exert little osmotic resistance, is possibly a relic of the past; perhaps also the synthesis of hippuric acid and the hydrolysis of urea (5); for none of these processes seem to have much value as functions of the mammalian kidney. In the bird absorption of fluid is, for the most part, effected in the cloaca, and the kidney may have preserved its secretory function. Some evidence of secretion of uric acid has recently been obtained in the fowl. If the view advanced here is a correct estimate of the position of secretory activity in the kidney, the value of phenol sulphone phthalein as a test for renal efficiency must be doubted; since this substance is apparently eliminated by secretion, and gives no evidence of functional capacity in dealing with ordinary waste products.

#### SUMMARY.

No intracellular concentration has been found to occur during excretion of urea and sulphate by the kidney. Evidence obtained by other observers that phenol sulphone phthalein is secreted shows that concentration of the dye takes place in the renal cells. It is therefore concluded that urea and sulphate are eliminated by a process other than secretion; and, in view of the relatively small increase in concentration which occurs in excretion of waste products as compared to dyes, this conclusion is extended to the urine as a whole. The conception of renal activity which justifies the employment of dyes in efficiency tests is probably erroneous.

Most of the expenses of this research have been covered by a grant from the Moray Fund of Edinburgh University.

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# ANTIDROMIC ACTION. Part I. BY J. N. LANGLEY.

(From the Physiological Laboratory, Cambridge.)

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OBSERVATIONS on vaso-dilatation caused by stimulating the posterior roots have been made almost entirely on the dog and frog, and almost entirely deduced either from a rise of temperature, or by increase of volume as shown by the plethysmograph. Morat(1), however, noted that a reddening of the skin could be directly observed. Most of his experiments were made on dogs. In young dogs he found that stimulation of the 6th or of the 7th lumbar posterior root caused flushing of pad, toes and of the adjoining skin. The 7th lumbar also caused flushing of the skin of the leg, whether a part or the whole is not mentioned. He stated that a similar result could be obtained in the cat and gave an experiment in which reddening of the pad and toes was caused by stimulating the 7th lumbar posterior roots. Bayliss(2) by the plethysmographic method did not obtain constant results in the cat, but in two cases he obtained increase of volume of the leg. The slight or absent result he attributed to a rapid loss of excitability of the roots. In the course of investigating whether the posterior roots had any effect on secretion of sweat in the cat(3) I noticed that flushing of the pad and toes of the hind foot, often intense, was produced in all the experiments by stimulating the posterior roots of the combined 6th and 7th lumbar nerves and that some effect could be obtained at the end of an experiment lasting several hours.

The fact suggested that by stimulating the 7th lumbar posterior roots after section of the sensory nerves near the periphery, the question whether the more central arteries are dilated by antidromic nerve impulses could be determined. The experiments of this nature led to others

on the effects of stimulating the peripheral nerves and of variations of arterial blood-pressure on capillary diameter which will be considered in a later paper.

*Method.* The cats were generally anæsthetised first with chloroform and then with chloroform and ether after tracheotomy; occasionally with urethane and C.E. A few, after being chloroformed, were decerebrated by Sherrington's method, and the anæsthetic discontinued. Two animals after decerebration were curarised. No notable difference was found by the different methods. The stimulus used was the interrupted current of an induction coil. Currents just felt on the tongue will produce marked flushing, but currents rather strong on the tongue give a better effect, especially towards the end of an experiment. From Stricker onwards nearly all observers have found mechanical stimulation to be more effective than electrical and some have not found electrical stimulation to have any effect. Mechanical stimulation of the nerve roots is effective on the cat's foot but does not cause greater flushing than electrical stimulation. I have not infrequently found towards the end of an experiment that it was less. In order to avoid as far as possible reflex effects in preparing the roots I passed a loose ligature round them, cut the roots with a single cut and then tied them. This always caused moderate flushing which only slowly lessened. In most cases stimulation was for about a minute at a time. The animals lay unfastened on a warmed table. During an observation the foot must not be moved, since the tint is readily increased by pressure on the veins.

For brevity I sometimes speak of flushing of the pad and of the hairless cushions of the foot as flushing of the foot. In the pad the larger central part I call the mid pad, the smaller eminences on either side of the mid pad I call the side pads. The 1st digit is rudimentary in the cat, so that the innermost toe is the 2nd.

Whilst the method of ocular inspection is excellent for marked changes of tint, it is not very satisfactory for slight and slow changes. Observation of a slight change would be much easier if one had a set of standard tints but I have not been able to obtain any which reproduce the varying tints of the skin with sufficient exactness. The opposite foot to that stimulated serves to a limited extent as a control. In some cases one can satisfy oneself whether the colour changes or not by stimulating several times. But even then one may still be doubtful. It is probable, however, that a change of blood supply which is not visible with certainty is of little importance. When there is marked flushing in one part of the foot, the rest of the foot may seem to become paler from the effect of contrast,

so that when the pallor is slight, it is advisable to cover the flushing parts, and to observe only those parts which apparently became paler.

1. *Flushing of the foot caused by stimulating the posterior roots.*

I have stimulated the posterior roots of the 7th lumbar nerve in more than thirty cats. In most cases the foot, *i.e.* the pad and toes, became a brilliant red. In others the flushing was moderate. In a few the flushing at first was doubtful; in these a distinct though slight effect was later obtained either by repeating the stimulation several times, or by increasing the strength of the stimulus. There are various factors in addition to the strength of the stimulus, which influence the depth of the flush; these are the height of the arterial blood-pressure, the thickness of the epidermis, and the responsiveness of the tissues. The responsiveness of the tissues to antidromic nerve impulses I think varies, since even in a cat in which the posterior roots have but a slight effect, the foot flushes well on washing it. The responsiveness appears to decrease with age, for in general the flushing was greater in young than in old cats. The flushing can be obtained repeatedly during an experiment lasting three or four hours, its intensity decreases, but it rarely fails entirely.

The time after the beginning of stimulation at which flushing begins to be visible varies. In favourable conditions it begins in about 5 seconds and reaches its maximum in 15 to 20 seconds, but after frequent repetition the change in tint is very gradual, and the maximum tint may not be obtained for 1 or even 2 minutes. The flushing subsides slowly; after an excitation lasting 30-60 seconds the full flush remains for a minute or two and slowly decreases in the subsequent 5 to 10 minutes. The slow subsidence of the dilatation was noticed by Bayliss in his plethysmograph experiments and was attributed by him to compression of the veins which the plethysmograph method is apt to produce. It is an advantage of the ocular inspection method that venous compression can be easily avoided.

Occasionally the 7th lumbar posterior roots cause slight pallor or no flushing in the inner side pad and 2nd toe, though the rest of the foot flushes, and this is more frequent when the flushing is slight. Further, when the flushing is slight, one stimulus may cause flushing in the whole foot, and another stimulus cause flushing in one part and pallor in another part; the paling part is usually the anterior part of the pad.

Bayliss found that excision of the lumbar sympathetic ganglia did not influence antidromic action. In one experiment I cut the grey rami of the 7th lumbar ganglion and stimulated the 7th lumbar posterior roots; there was no perceptible change in effect.

The striking fact about the posterior roots of the 6th lumbar nerve is that their effect is so small and variable. Out of sixteen experiments a marked antidromic effect was only obtained in four. In three of these the 2nd toe flushed and the others became slightly pale. The fourth experiment I shall speak of presently. In the other twelve experiments the effects were slight, or it was doubtful whether there were any. In most of them, it must be mentioned, the 6th lumbar (and 1st sacral) roots were stimulated after the posterior roots of the 7th lumbar—a condition not favourable for the detection of a slight change of tint. The effects were: doubtful pallor of the 2nd toe, slight general pallor, and occasionally slight general flushing.

The 1st sacral posterior roots had also a slight action, but they generally caused flushing of the 5th and 4th toes (rarely any of the 3rd toe) and slight pallor of the 2nd toe. The effect on the pad was variable, not infrequently a part or the whole became pale; the maximum extent of flushing found was of the outer side pad and the outer half of the mid pad. Sometimes there was slight pallor in all the toes, less frequently slight flushing in all. In one experiment only did the flushing caused by the 1st sacral overlap that of the 6th lumbar. In this (the fourth experiment mentioned above) the 6th lumbar posterior roots caused fairly good flushing in the 2nd toe, less in the third, slight in the 4th and pallor in the 5th toe. The 1st sacral posterior roots caused trifling but perceptible flushing, decreasing from the 5th toe to the 3rd, and pallor in the 2nd toe. The nerves in this case belonged to the anterior class, *i.e.* some of the fibres ordinarily in the 7th nerve were in the 6th lumbar nerve. The slight effects both of the 1st sacral and the 6th lumbar not infrequently vary with successive stimuli indicating a different balance of antagonistic factors. When the origin of the nerves is not markedly anterior or posterior, the 7th lumbar posterior roots have a greater effect than those of the 6th on the inner part of the foot, and a greater effect than those of the 1st sacral on the outer part of the foot, so that each part receives more antidromic fibres from the 7th lumbar than from any other nerve.

The 5th lumbar and the 2nd sacral roots, I have stimulated in two experiments only. The effect, if any, was slight pallor. I have not paid much attention to the web, but I have seen marked flushing in it on stimulating the 7th lumbar posterior roots after the hairs have been cut. The dorsal skin of the digits also flushes, but in the few observations I have made, the flushing was only slight. Thus antidromic vaso-dilatation though of varying degree can be seen in the whole area of the phalanges.

In the few observations made on the inner surface of the skin of the leg and on the superficial fascia, I have not found any distinct antidromic action. The pallor of the pad and toes caused by stimulating the posterior roots after section of peripheral nerves (cp. p. 439) shows, however, that there is some vaso-dilatation proximally of the pad.

As said above, the posterior roots of the 6th lumbar nerve whilst causing flushing in the inner toe or toes may cause pallor in the outer toe or toes, and the posterior roots of the 1st sacral may cause flushing in the outer toes and pallor in the inner toes. There is reason to believe that the 6th lumbar does not send afferent fibres to the outer toes, and that the 1st sacral does not send afferent fibres to the inner toes. The pallor, then, may provisionally be attributed to a fall of arterial pressure caused by dilatation of the vessels in the adjoining region, though the possibility of a reflex component must be considered later. On the same ground, the slight pallor in all the toes sometimes caused by the 6th lumbar and the 1st sacral roots may be attributed to dilatation proximally of the toes. Pallor may be produced in a more distant part, for when the 7th lumbar roots of one side cause marked flushing, it is accompanied by paling of the opposite foot; this is best seen by stimulating the roots of the two sides alternately at intervals of about five minutes.

Dilatation centrally of the pad besides tending to cause a fall of peripheral arterial pressure also tends to increase venous pressure. Increase of venous pressure readily causes capillary dilatation. Simply lifting the foot in any way commonly causes flushing of the pad and toes. When the cat is on its belly lifting the foot by a finger placed under the ankle, thus compressing the dorsal vein, may make the foot a brilliant red. If the 7th lumbar posterior roots are stimulated on one side, lifting each foot in this way when the flushing has nearly subsided generally causes the foot on the stimulated side to become much redder than that on the unstimulated side. The result strongly suggests that the stimulation has caused a decrease of capillary tone (cp. p. 441). I attribute, then, the slight flush of the whole of the foot which is occasionally produced by the 6th lumbar and 1st sacral posterior roots in cats having a median origin of nerves to a combination of increased venous pressure and decreased capillary tone. Sometimes on stimulating these roots one part of the foot will consistently become pale more than the rest in apparently identical conditions, indicating a difference in capillary tone in the different parts of the foot. There is one other point to mention. It is not uncommon for the flushing to increase after the stimulation has

ceased This may be due to earlier cessation of dilatation in some central part and consequent rise of local arterial blood pressure

The plan of the experiments was to cut one or more of the sensory nerves near the periphery and to observe the effect of the section on the flushing normally caused by stimulating the posterior roots Since, however, there are numerous nerve and artery anastomoses in the foot, it was necessary to make some observations on the extent of these in the cat

## 2 *The nerves and blood vessels of the foot of the cat*

The general features of the distribution of nerves and arteries in the foot of the cat are the same as those described in carnivora by Ellenberger and Baum, and in the dog by Sissons in their respective treatises on the Anatomy of Domestic Animals But there are one or two points, important for investigations on antidromic action, in which the anatomy in the cat differs from that they describe In the following account the anatomy is only given in so far as is necessary to understand the experimental results

On the dorsal surface of the foot the musculo cutaneous nerve (n peroneus superficialis) gives a nerve to each side of each digit The branch of the musculo cutaneous nerve which supplies the 2nd and 3rd digital nerves is joined by a branch from the anterior tibial nerve (superficial branch of n per profundus) The internal saphenous nerve commonly sends a small filament to the musculo cutaneous The point of division of the musculo cutaneous nerve in the metatarsal region varies, a common form is shown in the diagram, Fig 1

The internal and external saphenous nerves running respectively to the inner and outer surface of the foot sometimes appear to be connected with the adjoining musculo cutaneous branch, but I do not think they send fibres to the cushions of the toes Ellenberger and Baum state that in carnivora the anterior tibial nerve besides being connected with the musculo cutaneous branch described above is also connected with the two laterally placed musculo cutaneous branches (dotted lines in Fig 1) These I have not seen in the cat on dissection, but possibly small filaments are present

A diagram of the arrangement of the plantar nerves is given in Fig 2 The connections of the deep external plantar shown by dotted lines are variable, the filaments run parallel to the deep metatarsal arteries and may accompany them They run chiefly to deep structures and commonly send no fibres visible on dissection to the superficial plantar nerves But in one case—though in one foot only—the nerve near the central deep metatarsal artery was an obvious strand and was easily followed to the 4th digital nerve a little past its separation from the 5th digital The nerves for about .5 cm from the junction were treated with osmic acid



and teased, a few fibres ran to the 5th digital nerve and a small bundle ran backwards in the superficial plantar nerve. Thus the plantar digital nerves whilst usually arising from the superficial plantar nerves with little or no addition from the deep plantar nerves, may receive filaments from them.

Ellenberger and Baum call the branches of the external plantar nerve which run with the three deep metatarsal arteries the "common digital nerves," and say that they receive the superficial plantar nerves and then form the "proper plantar digital nerves 2 to 7." In the cat the "proper plantar digital nerves" are formed entirely or almost entirely by the superficial plantar nerves. Occasionally the deep external plantar sends a filament to join the superficial plantar nerve of the lateral surface of the 5th digit and there are no doubt other variations. The peroneal and posterior tibial nerves are each to be regarded as forming nerve plexuses in the foot, the strands of which separate at different points in different animals.

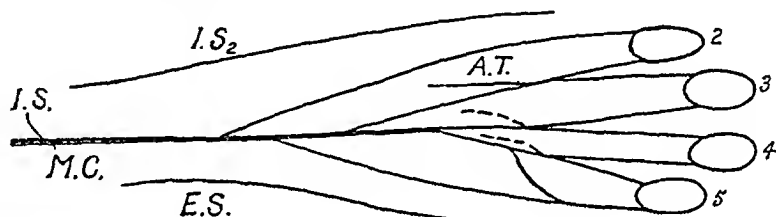


Fig. 1

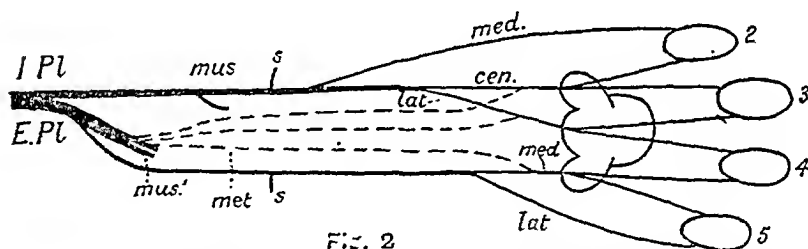


Fig. 2

Fig. 1. *I.S.* Internal saphenous nerve filament to musculo-cutaneous nerve. *I.S<sub>2</sub>*. Skin branch. *E.S.* Skin branch of external saphenous nerve. *M.C.* Musculo-cutaneous nerve. *A.T.* Superficial branch of anterior tibial nerve.

Fig. 2. *I.Pl.* Internal (medial) plantar nerve; *mus.* its deep (muscle) branch; *s.* its superficial (sensory) branch, dividing into medial, central and lateral branches. *E.Pl.* External (lateral) plantar; *mus.* its deep (muscle) branch, giving off three metatarsal filaments (*met.*) running near the deep metatarsal arteries (see Text); *s.* its superficial sensory branch dividing into a medial and a lateral branch.

I shall frequently have to speak of the effect of section (or of stimulation) a little proximally of the pad, of the several branches of the superficial plantar nerves. It is to be borne in mind that the medial branch of the internal plantar supplies the medial (inner) surface of the 2nd digit; the central supplies the adjoining surfaces of the 2nd and 3rd

digits and the inner side pad; the lateral branch supplies the adjoining surfaces of the 3rd and 4th digits and the mid pad, and that the medial branch of the external plantar supplies the adjoining surfaces of the 4th and 5th digits and the outer side pad, the lateral branch supplying the lateral (outer) surface of the 5th digit.

The course of the arteries was brought out by the starch injection method I have described elsewhere (This JOURN. 57. 1923, *Proc. Physiol. Soc.* p. lxx). Broadly speaking, the superficial structures of the metatarsal region are supplied with blood from the saphenous artery, by a small branch from the posterior tibial artery which runs with the plantar nerves, and by a small branch from the anterior tibial artery which runs with the musculo-cutaneous nerve, and, broadly speaking, the deep metatarsal structures and the digits are supplied with blood by the anterior tibial artery. The superficial arteries accompany the superficial nerves, dorsal and plantar, their communicating arteries will be mentioned presently. The statement of Ellenberger and Baum that the internal saphenous artery is the main source of the blood supply of the foot of carnivora is in the cat only true of the superficial structures.

The anterior tibial artery, after giving a branch to the tarsus, dips down between the 2nd and 3rd metatarsal bones as the perforating artery. The perforating artery gives off a branch which divides into several branches, one joins the artery of the external plantar nerve (communicating artery), one runs to the skin of the proximal metatarsal region. The perforating metatarsal gives off branches to muscles and divides into medial, central and lateral deep metatarsal arteries (Fig. 3 B). The muscle branches of these we need not notice. Near the end of the metatarsal region and beginning of the phalangeal region three small arteries (sometimes more) are given off (Fig. 3 A). The first gives off several branches, one joins the artery accompanying the underlying plantar artery (plantar communicating artery), another supplies the side pad, respectively the posterior part of the mid pad. The second gives off branches, one of which joins the artery accompanying the overlying musculo-cutaneous nerve (dorsal communicating artery). The third branch runs to the metatarsal region and the proximal part of the web, and in the case of the central deep metatarsal artery sends a branch to the anterior part of the mid pad. The occurrence of separate arteries to the proximal and distal portion of the mid pad accounts for the not infrequent difference in tint in the two portions on nerve stimulation. The general arrangement is shown in Fig. 3 A, but the point at which the arteries are given off varies in different cats. The course of the

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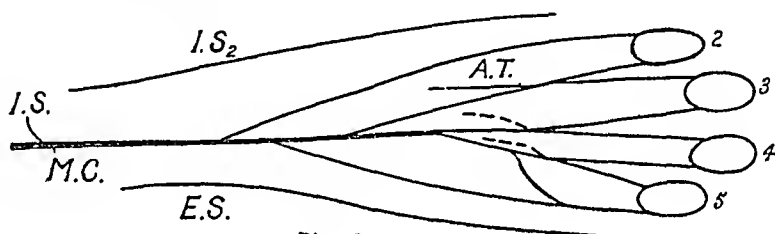


Fig. 1

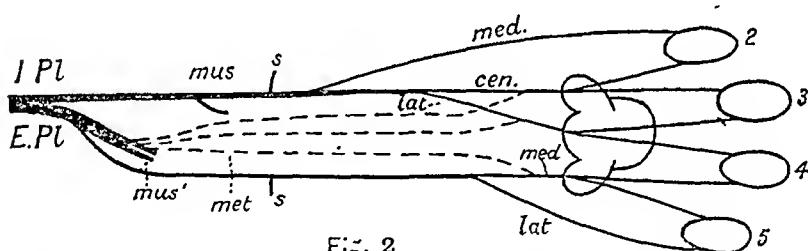


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(Fig. 3 *B* and *C*); the trunk passes under the tendon of the short flexor, crosses to the opposito side and sends small arterics to the web, claw and cushion and a centripetally running artery to the proximal portion of the digit and end of the metatarsal region (cp. Fig. 3 *B*).

As the plantar digital nerves do not run close to the digital arteries in the first part of their course it is doubtful whether they innervate any part except the branches and the end near the cushion of the toes.

When the central metatarsal artery divides into two digital arteries late in the web, there is near the point of division a small centripetally running artery given off near the point of division. It may be mentioned that small arteries anastomose in the skin and in the ventral surface of the tendon of short flexor muscle.

The plantar digital nerves send a branch accompanying the artery under the tendon, which forms there a bunch of Pacinian bodies, but I have not been able to trace any fibres crossing to the opposite side of the digit.

### 3. *Stimulation of posterior roots after section of peripheral nerves.*

From the anatomical course taken by the nerves it was clear that in order to ensure complete isolation of the vessels of the digits from possible nerve impulses when the posterior roots are stimulated, and thus to determine the effect of the roots on the central portions of the arteries, it would be necessary to cut not only the superficial plantar nerves a little proximally of the pad, but also the superficial dorsal, the deep branches of the external plantar, and possibly small branches of the anterior tibial nerves. The operation would cause so much disturbance of the digital circulation that it was not worth attempting.

The effects of stimulating the several peripheral nerves will be given in detail in a later paper, but I may mention here the general results. The superficial plantar nerves, on the first or later stimulation, always cause flushing, usually intense. Flushing produced by other peripheral nerves is slight and variable. The dorsal nerve of the foot has a very variable effect on the plantar region; it usually causes some primary pallor followed by slight flushing in patches, or occasionally general moderate flushing. The anterior tibial and deep external plantar generally cause marked pallor, but may cause slight flushing in patches. Each may cause a moderate after-flush. If any part of the flushing sometimes produced by these nerves is due to antidromic action, some variation in the result of stimulating the posterior roots after section of the plantar nerve branches would be expected.

The experiment which seemed most likely to give definite results was

to stimulate the posterior roots after section of one of the superficial plantar nerves. If no flushing was produced in the region supplied by the cut nerves, it might reasonably be concluded that the arteries centrally of the digital arteries did not dilate; on the other hand, if some flushing were produced, its degree could be compared with that of the region with uncut nerves.

*Section of the superficial branch of the internal plantar nerve.* In cutting this nerve, or other superficial nerves, the skin should be raised from the underlying tissue, since small veins lie immediately below the skin. It is not always possible to avoid cutting the small arteries which run with the nerves, but sometimes the main artery can be isolated. I have not found that it makes any difference whether the arteries are cut or not. The branches of the nerves were cut about 1.5 cm. proximally of the pad, and the 7th lumbar posterior roots stimulated. In four of the experiments the same striking results were obtained. Flushing was confined to the outer side pad, the 5th and 4th toes, and the rest of the foot became paler. Fig. 4 gives a diagram of the results.

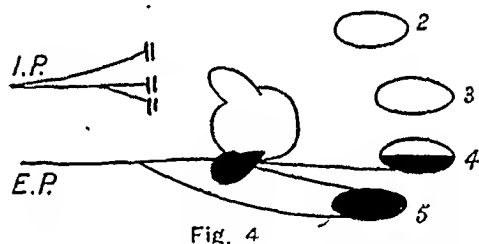


Fig. 4. Stimulation of the 7th lumbar posterior roots after section of the internal plantar nerve. The parts which flushed are black, the other parts became paler.

A noticeable feature which occurred in three of the experiments was the strictness of the limitation of flushing in the 4th toe to its lateral side. The medial side became paler and there was a narrow line between the flushed and pale halves. Towards the end of an experiment both flushing and pallor were less, and there was either slight flushing with no certain pallor, or slight pallor before flushing was perceptible. In these experiments it is clear that the flushing was produced by an action at the periphery. The dorsal arteries in their whole course and the other arterial trunks in their course proximally of the pad had their normal contingent of afferent fibres. Indeed it is not improbable that the innervation of the proximal part of the digital arteries was also intact, for, as we have seen, the plantar nerves only pass close to the digital arteries about the end of the 2nd phalanx.

In several experiments there was more or less variation from the results just mentioned. Sometimes the internal plantar region did not become pale, or not equally pale in all parts, and in one or two experiments, though not constantly, it flushed slightly. The results might have been partly due to a few antidromic fibres taking the course of the uncut nerves, it occurred however in two experiments in which the peroneal as well as the superficial internal plantar had been cut. In all these cases the 7th nerve had been stimulated before section of the superficial internal plantar and I take the absence of pallor or the slight flushing to have been caused mainly by increased venous pressure acting on capillaries decreased in tone by antidromic action.

*Section of both superficial plantar nerve branches* A few experiments only were made since the results were such as would be expected from the experiments already given. The posterior roots of the 7th lumbar nerve were cut and tied on both sides. On the side with plantar nerves intact, the stimulation caused the usual deep flush of the pad and toes. The stimulation on the side with cut plantar nerves usually caused paling of the foot. This varied in depth at different times in the course of the experiment, and was not always equal in all parts of the pad and in all the toes, and sometimes there was slight flushing in one or other part. The pallor was not as great as that usually present in the internal plantar region when the superficial internal plantar nerve alone was cut, i.e. when there was dilatation in the outer part of the foot.

*Section of digital nerves* In four experiments the plantar digital nerves were cut on both sides of the 3rd toe or 4th toe about half a centimetre proximally of the cushion and the posterior roots of the 7th lumbar nerve stimulated. The skin was cut in the mid ventral line of the toe, first being raised to avoid cutting small veins. It may be noted that these veins are apt to contract during the dissection. The plantar digital nerves are semi-transparent and not always easily distinguished from the surrounding connective tissue. In three such experiments, stimulation of the posterior roots of the 7th nerve caused pallor—in two cases considerable, in one slight but distinct—in the cushion of the toe the plantar digital nerves of which had been cut, and marked flushing in the rest of the foot. In these experiments the proximal portion of the digital artery had its nerves intact, and it is difficult to believe that if it had dilated it would not have caused flushing in the cushion of the toe. In the remaining experiment there was no marked change in the digit with cut nerves, sometimes there was slight pallor, sometimes slight flushing and generally some flushing after the stimulus had

ceased. In this case the dissection had caused protracted flushing of the toe.

*Compression of the toe to abolish conductivity in both plantar and dorsal digital nerves.* A string about 2 mm. in diameter was passed round the 3rd toe a little proximally of the 2nd phalanx and twisted. In a preliminary experiment the string was twisted and released, and about a minute later the superficial branch of the internal plantar stimulated. It was found that very strong twisting was required to prevent the nerve from causing a few drops of secretion<sup>1</sup>. The test is not an absolute one for the abolition of conductivity of antidromic fibres, since antidromic and secretory effects do not run parallel.

Three experiments were made in which the 7th lumbar posterior roots were stimulated after compression of the 3rd toe. The result was less striking and less constant than that after section of the nerves. The compression caused the toe to be bright red for a considerable time, indicating a protracted decrease of capillary tone. In each experiment the stimulation caused sometimes slight but distinct pallor in the 3rd toe, but not infrequently there was no perceptible change of tint, and sometimes there was slight flushing.

*Section of the plantar nerve running to one side of a digit.*

Most of the experiments mentioned above were made before I had found that the artery of one side of a toe crossed to supply the opposite side. This fact suggested another form of experiment, the nature of which will be best seen by an example. Fig. 5 is a diagram of the nerves cut, and of the course of the digital arteries.

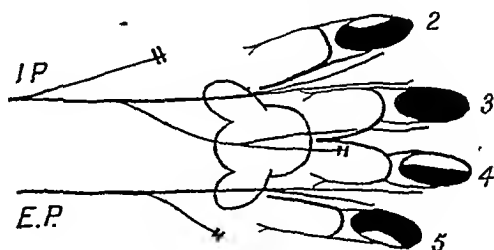


Fig. 5

The parts of the toes marked in black flushed on stimulating the

<sup>1</sup> As in all experiments in which the secretory effect is slight the nerve must be stimulated several times and with rather a strong current in order to be certain that no secretion occurs.

posterior roots of the 7th nerve<sup>1</sup>, the light parts became paler. The plantar nerves to the 3rd toe were intact and this flushed well. The plantar nerves to the central part of the artery of the 2nd and 5th toes were intact and the parts of the toes supplied by these parts flushed, but there was a pale patch on the opposite side supplied by the artery in its further course. The digital nerve on the medial side of the 4th toe was cut about the middle of its course and the half of the toe on this side became pale. The flushing in the 2nd and 5th toes was well over the median line, the pale area curved towards the free surface and extended to the base of the cushion, sometimes it could be seen to involve the adjoining part of the web. The area on the cushion, as seen from the side, was more or less oval. A pale area of varying extent on the side of the cushion opposite to that having the central part of the artery was found in similar experiments on the 3rd and 4th digits. The pale area is usually bounded at the proximal part of the cushion by a small vein, which runs obliquely towards the side of the digit which has the recurrent artery. If the digital nerve on either side of a toe is cut and stimulated, and a little later the 7th lumbar posterior roots are stimulated, there is usually flushing of both sides of the cushion indicating that the degree of spread of flushing from one side to the other is dependent, amongst other factors, upon the degree of capillary tone.

*Stimulation of posterior roots after clamping the abdominal aorta.*

The facts given above are, I think, only explicable on the theory that capillary dilatation is the chief factor, if not the only one, in antidromic flushing. If antidromic action decreases the tone of the capillaries stimulation of the posterior roots should cause some flushing in the absence of arterial pressure, provided there is some venous pressure. In order to determine whether this effect is produced, the abdominal aorta was clamped, and when the feet had become quite pale, the posterior roots of the 7th lumbar nerve were stimulated on one side. The stimulation caused slight, but distinct, flushing of the pad and toes, the opposite side remaining pale. In the conditions of the experiment dilatation of arteries would tend to take blood from the capillaries, and in consequence I consider the flushing to have been due to a decrease of capillary tone which allowed them to be slightly distended by the venous blood-pressure.

*Remarks.* The preceding results give, I think, good ground for

<sup>1</sup> The 1st sacral posterior roots caused flushing in the 5th and 4th toes like that caused by the 7th lumbar roots but pallor in the 3rd and 2nd toes.



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*Section of the plantar nerve running to one side of a digit.*

Most of the experiments mentioned above were made before I had found that the artery of one side of a toe crossed to supply the opposite side. This fact suggested another form of experiment, the nature of which will be best seen by an example. Fig. 5 is a diagram of the nerves cut, and of the course of the digital arteries.

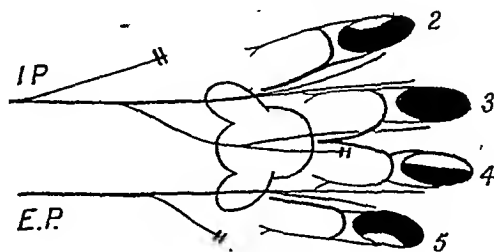


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The parts of the toes marked in black flushed on stimulating the

<sup>1</sup> As in all experiments in which the secretory effect is slight the nerve must be stimulated several times and with rather a strong current in order to be certain that no secretion occurs.

dilatation, but the responsiveness of the tissue affected by antidromic nerve impulses may be much less than that of the sweat glands to sympathetic nerve impulses and the occurrence of flushing may be to some extent counteracted by dilatation proximally of the pad.

### SUMMARY.

The experiments were made on the cat.

1. Stimulation of the posterior roots of the 7th lumbar nerve with induction currents causes flushing in the whole of the skin of the digital region of the hind foot. In the pad and the cushions of the toes the flushing is usually intense and detailed observations were confined to these parts. The flushing was first observed by Morat. The 6th lumbar and the 1st sacral posterior roots in nearly all cases cause much less distinct flushing, and in all the cases observed it was in a part of the foot only. The 6th lumbar generally causes flushing in the 2nd toe and inner side pad, the 1st sacral in the rest of the foot, but the area affected varies somewhat with the origin of the nerves from the spinal cord. In one experiment in which the origin was anterior the areas of the two nerves overlapped. The area which does not flush usually becomes paler. Sometimes neither nerve has any certain effect, this is more frequent with the 6th lumbar than with the 1st sacral posterior roots and both may cause slight pallor in the whole foot. The areas affected by the posterior roots of the several nerves correspond closely with the cutaneous areas of the nerves described by Sherrington.

2. The sympathetic secretory fibres which run to the 6th lumbar and 1st sacral nerves caused marked secretion in areas which are apparently not supplied with posterior root fibres from these nerves, suggesting that the sympathetic fibres spread out in the lumbo-sacral plexus more than do the posterior root fibres of the several spinal nerves.

3. Nearly all the antidromic vaso-dilator fibres to the pad and cushions of the toes run in the superficial branches of the plantar nerves, a few take a variable course by one or more of the other nerves. The effect of these is slight and inconstant and generally in small patches. From observations on the effect of stimulating the 7th lumbar posterior roots after section of one or more of the nerves near the periphery it is shown that the direct course of the arteries supplying the cushions of the toes up to the small branches given off by the digital arteries are not perceptibly affected by antidromic impulses, and it is concluded that they are unaffected. One form of experiment depends on the fact that one side of each toe is supplied by an artery which crosses from the

sample of blood was taken when the animal showed obvious symptoms, as it was found that the condition of shock during convulsions prevented the requisite amount of blood being obtained within a reasonable time. Though the injection of glucose enables the animal to recover and appear normal, this condition of shock apparently persists for a considerable time, for the same difficulty was experienced in obtaining the necessary blood even many hours after the convulsions.

As will be seen from the table of results, there is a sharp fall in the inorganic phosphorus within the first hour, the values obtained then and at the time of convulsions showing little change. The average fall in the inorganic phosphorus is about 30 p.c. The few results which we have been able to obtain during recovery, indicate that the value for the inorganic phosphorus rises only very gradually to its original level, and in some cases has not done so by the next day. It seemed possible that the fall in the inorganic phosphorus might be due to the formation of an organic complex; but estimations of the total acid-soluble fraction do not tend to support this view. For example it will be seen that in the case of rabbits 11 and 15, though there is a slight fall in the total acid-soluble phosphorus, this corresponds approximately with the fall in the inorganic phosphorus. Hence the organic fraction is apparently unchanged. But since the differences in the values for the total acid-soluble fraction lie within the experimental error of the method, the point cannot be regarded as definitely established. A micro-Neumann incineration method was used, the probable error of which is 3 p.c. If the inorganic phosphorus which disappears after insulin is the only phosphorus taking part in the formation of a complex, then it is clear that the question cannot be decided with the methods at present available.

*Discussion.* The fall which we have observed in the inorganic phosphorus may be due to one of several reasons.

The phosphorus may have contributed to the formation of an organic complex in the blood. If this is not of an acid-soluble type (though our results do not definitely exclude the possibility), it may yet be of such a nature that it is precipitated with the proteins; if this were the case it would not be estimated.

The injection of insulin may have led to an increased excretion of phosphorus in the urine. We have been unable to obtain samples of urine over short periods for the investigation of this point. Two of us however (V. B. W. and C. E. W.) in unpublished experiments on the phosphate excretion in man have never found an increased phosphorus elimination associated with a fall in the blood phosphate.

There remains the possibility that the phosphorus is taken up by the tissues. Two of us<sup>(8)</sup> have suggested that though glycogen disappears after insulin, the carbohydrate content of the body may be very little affected, and that glycogen is converted into some sugar complex which is resistant to acid hydrolysis. It is not unlikely that in this conversion phosphorus may play an essential part. It has been shown<sup>(9)</sup> that the nature of the blood sugar of persons suffering from diabetes mellitus is changed after injection of insulin. We are at present engaged in testing the possibility of an alteration in the blood phosphate as a result of administration of insulin to clinical cases.

#### SUMMARY.

1. Injection of insulin into rabbits causes a rapid fall in the inorganic phosphorus of the blood.
2. This low level is maintained for many hours after recovery from convulsions by glucose.
3. The total acid-soluble phosphorus is very little altered.

We wish to thank Professor F. G. Hopkins for the interest he has taken in this work.

The expenses of this research were in part borne by grants made by the Scientific and Industrial Research Board (to W. S., V. B. W. and C. E. W.) and by the Medical Research Council (to L. B. W.).

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## PROTOCOLS.

## Normal rabbits

	Time	Blood phosphate mg P %		Time	Blood phosphate mg P %	
R. 2	10-00	5.44	R. 4	11-00	6.68	
	10-10	Injected with saline		11-15	Saline injection	
	12-00			5.39	12-30	6.71
	2-35			5.38	3-30	6.47
R. 5	10-15	4.11				
	10-45	Saline injection				
	12-00	4.32				
	3-55	4.37				

## Insulin rabbits

R. 3	9-40	5-81	R. 6	10-00	5-72		
	10-00	Insulin		10-15	Insulin		
	11-00	3-05		11-30	4-73		
	12-00	3-26		2-00	4-26		
	12-25	Convulsions relieved by glucose			Convulsions did not occur		
	3-30	3-40					
	next day	4-74					
	Time	Inorganic P.	Acid-sol. P.		Time	Inorganic P.	Acid-sol. P.
R. 8	10-00	4-55	—	R. 11	10-00	4-05	41-1
	10-20	Insulin	—		10-15	Insulin	—
	11-15	2-05	—		11-20	2-50	40-7
	2-30	3-18	—		1-00	2-55	—
	—	No convulsions occurred	—		1-15	Convulsions	—
	—	—	—			Glucose given	
	—	—	—		5-00	3-10	—
R. 15	9-45	6-23	42-3	R. 10	10-00	6-74	—
	10-00	Insulin	—		10-15	Insulin	—
	11-35	4-94	40-0		11-50	4-26	—
	11-45	Convulsions	—		1-30	Convulsions	—
		Glucose given				Glucose given	
	2-30	4-72	40-3		4-30	4-30	—
	6-00	5-20	—		—	—	—
R. 14	10-00	7-76	—	R. 17	10-15	4-44	—
	10-10	Insulin	—		10-30	Insulin	—
	11-30	6-36	—		11-30	Convulsions	—
	11-35	Convulsions	—		11-35	2-80	—
	—	Apparent recovery by glucose	—		—	Glucose	—
	—	—	—		2-00	2-94	—
	2-20	6-11 convulsions.	—		5-45	3-31	—
next day	6-78	—	—	—	—		

## THE RELATIVE IMPORTANCE OF THE FACTORS CONCERNED IN THE FORMATION OF THE URINE.

By N. B. DREYER, *Sharpey Scholar*, AND E. B. VERNEY, *Beit Memorial Research Fellow*.

(From the Institute of Physiology, University College, London.)

In a paper already published (Starling and Verney<sup>(1)</sup>) it was shown that the isolated mammalian kidney, perfused by means of a heart-lung preparation, was capable of secreting a fluid hypotonic to the blood plasma, and of concentrating urea and glucose. In this preparation there are three independent means by which a variation in the rate of secretion is possible. These are: the pressure at which the blood is supplied to the kidney, its velocity of flow, and its chemical composition. An attempt has been made to relegate to each of these factors its proper share in the process of secretion by the kidney. By keeping one factor constant and noting the effect of a variation in the second on both the third factor and the rate of urinary flow, it was possible to estimate in turn the relative importance of all these factors. We will first describe experiments in which the composition of the blood was maintained constant.

Some evidence was brought forward previously in favour of the height of the renal arterial pressure, rather than the rate of blood flow, being the more important factor in the causation of the urinary flow. A more extended use of the method has inclined us still more to this view.

When the artificial circulation is turned on, the blood flow through the kidney is at first very small. This gradually increases up to a maximum for the particular pressure employed, and coincident with this recovery the urinary flow begins. This period of recovery can be shortened appreciably by section of the corresponding splanchnic nerve a considerable time before the excision of the kidney. After the preparation has reached a steady state, any alteration in the pressure at which the blood is supplied to the kidney is accompanied invariably by a change in the rate of urinary flow, and usually by a change in the rate of blood flow.

Often the rate of urinary flow on the one hand and the rate of blood flow and blood-pressure on the other show a linear relationship. Such

a case is charted in Fig. 1. Although in this experiment an almost direct proportionality holds between both rate of blood flow and blood-pressure

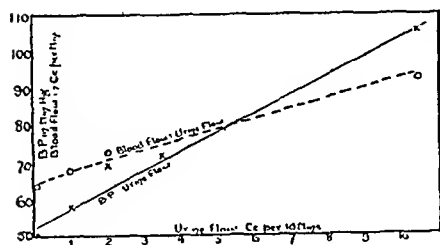


Fig. 1.

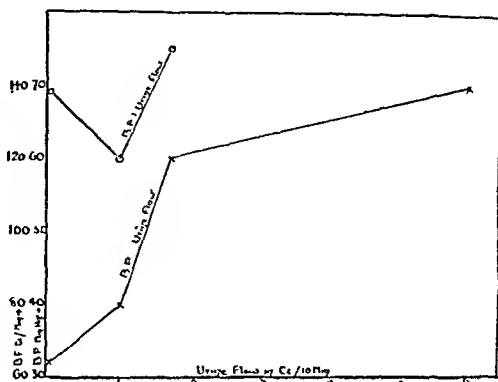


Fig. 2.

and rate of urinary flow, it will be seen from the slopes of the curves that percentually the relationship between blood-pressure and urinary flow is a closer one than that between blood flow and urinary flow. The blood-pressure readings are measured throughout, at the renal arterial cannula. Sometimes, however, the relations are not so close and in these cases the rate of blood flow shows a greater tendency to wander from the narrow path of linear relationship with urinary flow, than does the height of arterial pressure.

In Fig. 2 are some readings taken from an experiment in which the conditions were as steady as possible. Although a rise or fall in blood-pressure was invariably accompanied by a change in rate of urinary flow in the same sense, the alteration in blood flow might even be in the opposite sense. Thus lowering the blood-pressure from 40 to 32 mm. Hg was accompanied by a diminution in the rate of urinary flow from 1 to 2 c.c. per 10 mins., but by an actual increase in blood flow from 60 to 69 c.c. per min. This is brought out even more clearly in the following two charts taken from two further experiments. In Fig. 3 the rate of urinary flow is a linear function of the blood-pressure whilst being quite unrelated to the rate of blood flow. In the experiment from which Fig. 4 is taken, raising the blood-pressure from 50 to 70 mm. Hg caused an increase in blood flow from 120 to 150 c.c. per min., and in urine flow from 2.6 to 11 c.c. per 10 mins. Lowering the blood-pressure to 42 mm. Hg on the other hand, was accompanied by a fall in urine flow to 2.4 c.c. per 10 mins., whilst occasioning no alteration in the rate of blood flow. It is important to remark that during the period over which these readings

were taken, no alteration was actively made in the composition of the circulating blood.

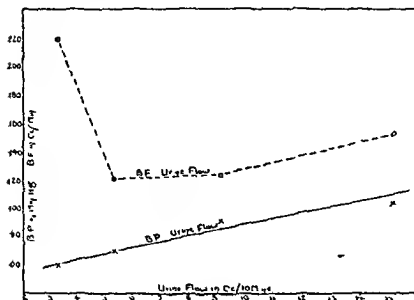


Fig. 3.

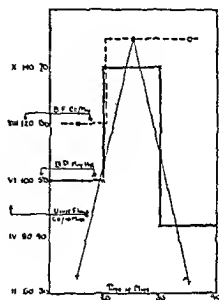


Fig. 4.

The experiments are confirmatory of the results of Richards and Plant who showed(2) that changes in the renal blood-pressure were accompanied by parallel changes in the rate of urinary flow although the velocity of blood flow through the kidney was maintained constant.

We now pass to a consideration of the influence exercised by a change in the composition of the blood on the rates of blood flow and urine flow respectively, the blood-pressure being maintained constant. For this purpose the effects produced by the addition of solutions of sodium chloride, urea, and sodium sulphate have been studied.

(a) *Sodium chloride.* 0.9 p.c. NaCl added to the circulating blood invariably gives rise to a well-marked diuresis, and this may be accompanied by no change in the rate of blood flow or by a rise or fall. This last accompaniment is not infrequent, which proves that here again velocity of blood flow per sec. is not a pre-important factor.

Fig. 5 is from an experiment in which the addition of normal saline caused a well-marked diuresis unaccompanied, however, by any change in the rate of blood flow. It will be seen that on the addition of 200 c.c. of saline the urinary flow increased from 3.8 to 8.4 c.c. per 10 mins. Lowering the arterial pressure from 140 to 98 mm. Hg did not prevent the urinary flow increasing still further to 10.6 c.c. per 10 mins. No change in the rate of blood flow was demonstrated. A further fall in the blood-pressure, however, was sufficient to lower both the rate of urinary flow and that of blood flow.



have shown that changes in the composition of the blood are also pre-eminently important in this direction when the blood-pressure remains constant, being often unaccompanied by any change in the velocity of blood flow. We are therefore forced to the conclusion that the two physico-chemical factors which are of primary importance in the causation of

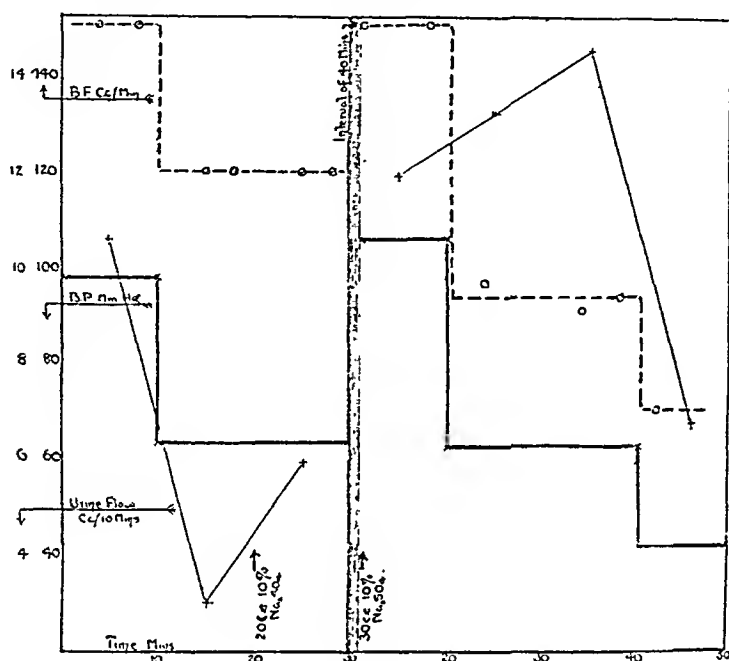


Fig. 9.

the flow of urine are (a) the height of the arterial blood-pressure and (b) the chemical composition of the blood. Blood flow is a necessary accompaniment of secretion but is not to be regarded as a cause *per se*.

We wish to express our indebtedness to Professor Starling for his help and advice throughout this work.

The expenses of this research were defrayed out of a grant from the Government Grant Committee of the Royal Society.

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THE INFLUENCE OF OXYGEN SUPPLY ON THE  
RESPONSE OF THE ISOLATED INTESTINE TO  
DRUGS. BY LOUIS GROSS<sup>1</sup>, *Beit Memorial Research Fellow*,  
AND A. J. CLARK.

(*From the Pharmacological Laboratory, University College, London.*)

THE isolated intestines of rats and rabbits were used in the following experiments. These were suspended in the usual manner in Tyrode's fluid. We found that cutting off the oxygen supply caused a lowering of the tonus of the gut although the pendulum movements continued unaltered, and that with the loss of tone the gut ceased to respond to adrenalin and pilocarpine.

A stream of air maintained the activity of the gut as well as a stream of oxygen, but the effects of cutting off the oxygen or air were not due to any mechanical effects, for the substitution of oxygen by a stream of nitrogen (Fig. 3) produced exactly the same effects as did the simple cutting off the oxygen supply (Fig. 1). When the supply of air or oxygen to the rat's or rabbit's gut is cut off, there is at first a slight rise in tonus and then a rapid fall. When the air is turned on, there is a slow rise of tonus (Fig. 2).

When the gut is relaxed by the lack of oxygen it is very insensitive to the action of adrenalin. Fig. 2 shows that adrenalin 1 in 25 million, which produces a strong effect on the normal gut, produces no effect on the relaxed gut. Fig. 5 shows that as the gut regains its tonus after oxygen lack it becomes progressively more sensitive to adrenalin.

The failure of the asphyxiated gut to respond to adrenalin might be due to its being in a state of maximum relaxation but the gut is equally insensitive to the action of stimulants such as pilocarpine, as is shown in Fig. 4. This figure shows that pilocarpine in moderate concentrations does not act on the asphyxiated gut, but that as soon as air is introduced the pilocarpine acts and the gut contracts violently. The asphyxiated gut is not paralysed for it can respond to the action of stimulants acting directly on the muscle. Fig. 1 shows this in the case of potassium chloride, and Fig. 6 shows the same effect with barium chloride.

<sup>1</sup> Aided by grants from the Medical Research Council and British Medical Association, London.

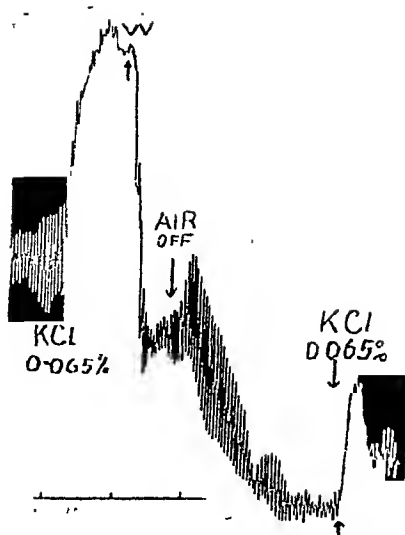


Fig. 1.

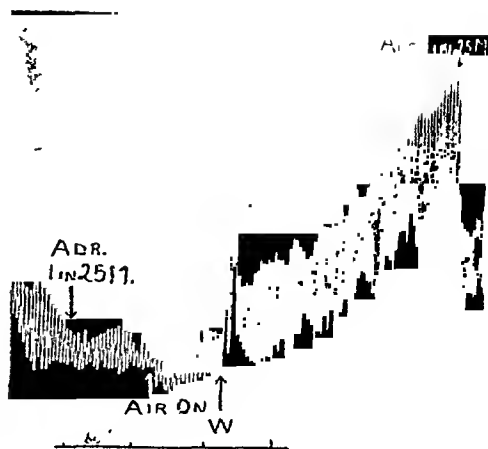


Fig. 2.

Fig. 1. Isolated gut of rabbit. Effect of increasing concentration of KCl from .02% to .065%. (1) Gut supplied with oxygen; (2) on asphyxiated gut.

Fig. 2. Isolated gut of rabbit deprived of oxygen for a few minutes. Action of adrenalin (1) on asphyxiated gut, (2) on gut supplied with air.

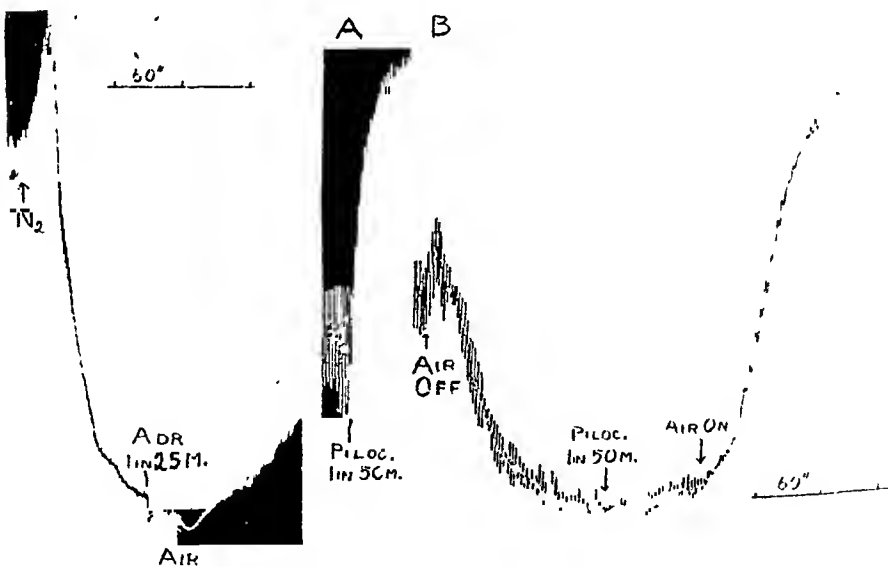


Fig. 3.

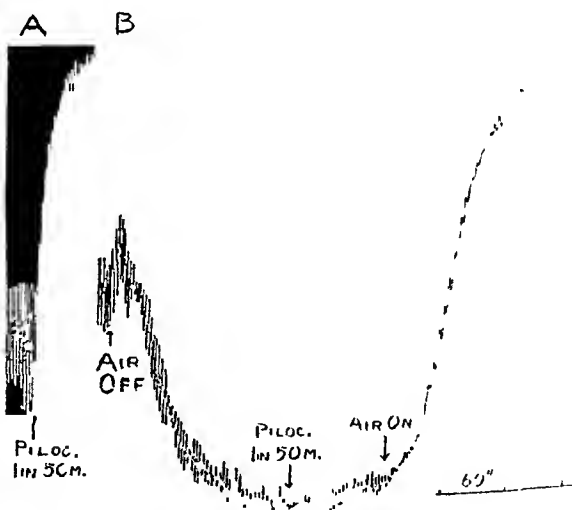


Fig. 4.

Fig. 3. Isolated gut of rat. Effect of replacing air by nitrogen, and action of adrenalin on asphyxiated gut.

Fig. 4. Isolated gut of rat. Effect of pilocarpine: A. on gut supplied with oxygen; B. on asphyxiated gut.

Asphyxia therefore appears to paralyse the sympathetic and parasympathetic nerve endings in the gut long before the gut muscle itself is paralysed. This result agrees with the work of Gunn and Underhill (1) who showed that the isolated gut, when kept for long periods, first became insensitive to parasympathetic stimulants, then to sympathetic stimulants and last of all to plain muscle stimulants. These workers also showed that the pendulum movements continued long after the nerve endings had become paralysed but that they ceased at the same time as the gut became insensitive to direct muscle stimulants. They adduced these facts as evidence of the myogenic origin of pendulum movements and our results support the same view. An examination of our tracings shows that the pendulum movements of the gut are not altered either in rate or extent at a stage in asphyxia when the gut no longer responds to either adrenalin or pilocarpine.

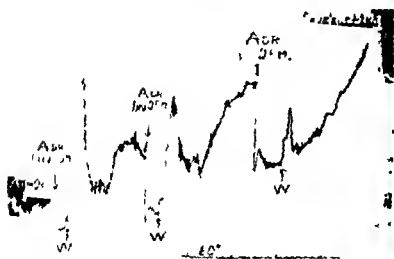


Fig. 5.



Fig. 6.

Fig. 5. Isolated gut of rat deprived of oxygen for a few minutes. Action of adrenalin with gradually increasing air supply.

Fig. 6. Isolated gut of rat. Action of barium on gut: A, when supplied with oxygen, B, when deprived of oxygen. The initial tonus of the gut in B was much lower than the initial tonus in A.

This paralysis of specific nerve endings by asphyxia was not observed in the other isolated organs tested, namely the uterus and heart. For example the isolated auricle of the rabbit is fairly rapidly affected by lack of oxygen, the height and rate of the contractions being diminished, and this effect is produced even more rapidly if nitrogen is substituted for oxygen. The asphyxiated auricle, however, responds to adrenalin

and pilocarpine in the same manner as does the normal tissue. The effect produced on the gut by lack of oxygen appears therefore to be peculiar to this tissue. Apparently there are in the isolated gut two independent mechanisms, one regulating the pendulum movements and the other regulating the tonus. The latter mechanism is intensely sensitive to the action of adrenalin and pilocarpine for these drugs alter tonus in concentrations far lower than those required to alter pendulum movements (Alvarez(2)). Lack of oxygen paralyses the tonus regulation mechanism very rapidly and hence renders the gut insensitive to low concentrations of the above mentioned drugs.

Evidence from other isolated tissues suggests that the sympathetic and parasympathetic nerve endings are not particularly sensitive to lack of oxygen. The effects observed with the gut can, however, be explained on the assumption that the tonus of the gut is maintained by the nerve cells in Auerbach's plexus: for nerve cells are known to be very sensitive to oxygen lack. The results in this paper support the view that the pendulum movements on the other hand are myogenic.

#### CONCLUSIONS.

(1) Cessation of oxygen supply, or the substitution of nitrogen for oxygen, causes an immediate loss of tonus in the isolated intestine.

(2) Lack of oxygen does not affect the pendulum movements of the gut for a long time.

(3) Lack of oxygen causes in the isolated gut a great diminution in sensitivity to adrenalin and pilocarpine, but no corresponding diminution in the reaction to direct muscle stimulants such as potassium and barium.

(4) These results support the view that the pendulum movements of the gut are myogenic, but suggests that the tonus of the gut is neurogenic.

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# ON THE ACTION OF PHLORHIZIN ON THE KIDNEY. By E. B. MAYRS.

*(From the Department of Pharmacology, Edinburgh.)*

GLUCOSE is the most important diffusible substance in the blood which is completely held back by the normal kidney. On Cushny's theory (1) of kidney action, the glomerulus and capsule are completely permeable to glucose, but the glucose does not pass into the urine because it is all absorbed by the tubule cells. Strong evidence for this view is afforded by Wearn's demonstration (2) that in the frog injection of glucose is followed by the appearance of a reducing substance in the glomerular filtrate, though none may be present in the urine. A similar conclusion is arrived at by Clark (3) from perfusion experiments on the frog's kidney. Further, in the albuminuria occurring in renal disease, proteins can sometimes be shown to have passed through Bowman's capsule (4), and yet no glucose appears in the urine. It is difficult to see how the highly diffusible glucose can in such cases fail to reach the urine unless it is absorbed by the renal tubules. The diffusibility of sugar would also lead us to expect that injury to the kidney might cause the appearance of glucose in the urine, but the only condition in which this occurs from renal changes is under poisoning with phlorhizin, in which there is reason to believe that the tubule cells are the site of action; if this consists in hindered absorption by them, the concentration of glucose by the kidney after administering phlorhizin might approach that of a "no-threshold" substance (1), but could not exceed it. It seems desirable then to determine how far this occurs, and I have made some experiments on rabbits. Sulphate was chosen as the standard "no-threshold" body, in preference to employing the normal urea of the blood; because in the rabbit sulphate (which is practically non-toxic) is more completely "no-threshold" in character than urea (5); and intravenous injection of sodium sulphate has the additional advantage of ensuring the diuresis necessary for short experiments. A similar method of investigation could be used for human subjects, and for that purpose urea, creatinine, or phosphate could be selected as the standard for comparison, and no injection would be necessary.

The rabbits were given varying amounts of phlorhizin subcutaneously, in warm saline or dilute alcohol. The quantity injected was always fairly

has produced sufficient hyperglycæmia (about 0.5%) to make the kidney excrete glucose almost as effectively as if it were under the influence of phlorhizin; though with less diuresis and more time for re-absorption this might not have occurred. The narcotic hyperglycæmia of the first two experiments has not had this effect. It may be simply a question of degree, or perhaps part of the normal blood sugar exists in a form different from that of injected glucose (7), which may act to some extent as a foreign body.

Previous investigations have shown that urea is less efficiently concentrated than sulphate by the rabbit's kidney (5). When a mixture of these substances is excreted, the urine/plasma ratio of sulphate is higher than that of urea. It was found to be from 1.50 to 2.75 times as high in different experiments, and the average was 2.01 times. It is therefore necessary for the stability of the absorption theory to suppose that some urea is re-absorbed from the tubules by their lining epithelium, and this point should be considered in deciding the value of renal efficiency tests based on the concentration of urea by the kidney. Since the response of the kidney to phlorhizin has also been used as a test for renal efficiency, it is of interest to see whether there is any theoretical advantage in employing this method. In pathological conditions of the kidney urea is often held back until it reaches a high level in the blood, and in these cases its concentration in the urine is low. The efficiency of excretion may be judged by comparing the percentages of urea in the plasma and urine, and observing the extent of their departure from the normal. On the absorption hypothesis urea retention is probably not due to inability of urea to reach the tubules, since the larger molecule of albumen can pass through the glomerular membrane, but to inability of the damaged tubule cells to avoid re-absorbing some of the urea in an attempt to concentrate it by removing water against its osmotic resistance. Phlorhizin has been found to produce less glycosuria in cases of nephritis (8), and has been used as an efficiency test on the ground that its effectiveness in causing excretion of blood sugar depends on the integrity of the renal epithelium. This is analogous to the retention of urea. After phlorhizin glucose should behave in much the same way as urea, and if the latter can diffuse back from the tubules into the blood the same assumption may be applied equally to glucose.

In order to demonstrate the superiority of the phlorhizin test it would be necessary, in the first place, to show that when acting on the normal kidney phlorhizin can render glucose more completely "no-threshold" in character than urea ordinarily is; and, secondly, to show that a dose

large enough to produce a fairly constant effect in health would not be injurious to the subject of the test. Evidence has been obtained that a rabbit fully under the influence of phlorhizin can excrete glucose slightly better than urea. The concentration ratio of urea was determined in Exp. 12, and was found to be 2.45. The corresponding glucose concentration ratio was 2.95. This difference is not of much importance, and as the normal human kidney is probably better able to concentrate urea than that of the rabbit, one cannot conclude that there is any material advantage in using the phlorhizin test. As regards the question of dosage it can only be said that 0.1 grm. per kilo subcutaneously was not found sufficient to produce the maximum effect in the rabbit, and that this dose is relatively very much larger than has been given to human subjects undergoing a renal efficiency test. Hence, unless the drug is given in excessive amounts, one cannot be quite sure that the kidney is in all cases under its influence to the same degree; and if too small a dose were employed, even the healthiest kidney could not be expected to concentrate glucose very much.

#### SUMMARY.

A procedure has been described for measuring the effect of phlorhizin on the kidney of animals. It is suggested that a similar method might be used for determining the efficiency of the human kidney in excreting any constituent of the urine; provided that one of the "no-threshold" substances is known to be excreted in a normal manner. The method described avoids uncertainty as to whether the concentrations observed have been influenced by diuresis, since both the standard and the substance being examined are affected to the same extent.

The nature of the renal mechanism for the retention of glucose has been discussed, and it has been shown that phlorhizin does not usually enable the kidney to excrete glucose quite as effectively as "no-threshold" substances.

The use of phlorhizin for renal efficiency tests has been criticised, and the dose usually given has been shown to be quite inadequate.

All the results obtained are in accord with the absorption theory of the excretion of urine.

The expenses of this research have been defrayed for the most part by a grant from the Moray Fund of Edinburgh University.





# PROCEEDINGS

## OF THE

# PHYSIOLOGICAL SOCIETY,

*October 21, 1922.*

**The U-Tube manometer test with relation to muscular exercise.**

By W. D. HAMBLY and B. A. McSWINEY<sup>1</sup>.

In 1920<sup>2</sup> Flack published an account of the U-Tube manometer test as one method of estimating the physical efficiency of Air Force pilots

A modification of Flack's experiment was used for testing individuals before and after muscular exercise. The pulse rate was taken for one minute, a column of mercury was raised by expiration to a height of 40 mm, and held there for twenty seconds, then the pulse rate was again taken for a minute

For convenience in comparing the results of different individuals the difference between the two pulse rates was calculated as a percentage

Tests were made every thirty minutes on men resting in the intervals between the tests. On the following day heavy muscular work (weight carrying) was performed in the same intervals

	Rested Subjects	Worked Subjects
No change of pulse rate after test	16.1 p.c. of cases	1.4 p.c. of cases
Rise of pulse rate after test	34.8 " "	0.7 " "
Fall of pulse rate after test	49.1 " "	97.9 " "

The above figures are based on 200 tests taken on thirty men, resting or doing the same work. It will be observed that in resting men a rise and fall occur indifferently but that after muscular exercise there is almost invariably a fall of pulse rate. After rest the fall in the pulse rate produced by the test has an average value of 0.5 p.c. After exercise the fall has an average value of 10 p.c. The latter fall is considerably greater than the probable error of the mean value and certainly represents an average physiological change produced by exercise. The results of the test are fairly constant for a given individual under similar conditions but vary in amount and character in different individuals. Observations in factories showed similar results

<sup>1</sup> Working for "Industrial Fatigue Research Board"

<sup>2</sup> Flack, M. *Med Res Council* 1920 Spec Rep Ser 23

# PROCEEDINGS

## OF THE

### PHYSIOLOGICAL SOCIETY,

November 18, 1922.

#### The rickets-producing effect of dried thyroid.

By EDWARD MELLANBY.

I have shown elsewhere. (*Medical Research Council*, Special Report Series No. 61) that thyroid gland given by mouth to puppies has no antirachitic effect when added to a rickets-producing diet, although its stimulating influence on the metabolism is great. In more recent experiments this substance has been added to diets usually of a borderline nature which alone caused in some cases slight rickets, in other cases no rickets. The additional dried thyroid increased the rachitic condition.

In the following series the dietetic constituents common to all were separated milk powder 7 grams, meat 10 grams, bread 50-150 grams, butter 10 grams.

No. of Exp.	Thyroid (dried)	$\text{CaH}_4(\text{PO}_4)_2$ , $2\text{H}_2\text{O}$	Duration	Gain in weight, gms.	Radiographic result
{ 497	0.33 g.	1.0 g.	13 weeks	1600	Rickets }
{ 501	0	1.0 g.	13 "	1930	Normal }
{ 498	0.33 g.	0	13 "	1240	Rickets }
{ 499	0	0	13 "	1950	Practically normal }

In the following series separated milk powder 15 grams, meat 20 grams, lard 10 grams, orange juice, yeast and sodium chloride were eaten by all animals.

No. of Exp.	Thyroid	Bread	Duration	Gain in weight, gms.	Radiographic result
{ 468	0	180 g.	12 weeks	3450	Slight rickets }
{ 469	0.33 to 1.32 g.	180 g.	12 "	2080	Bad rickets }
{ 481	0	90 g.	12 "	1760	Practically normal }
{ 479	0.33 to 1.32 g.	90 g.	12 "	1240	Bad rickets }

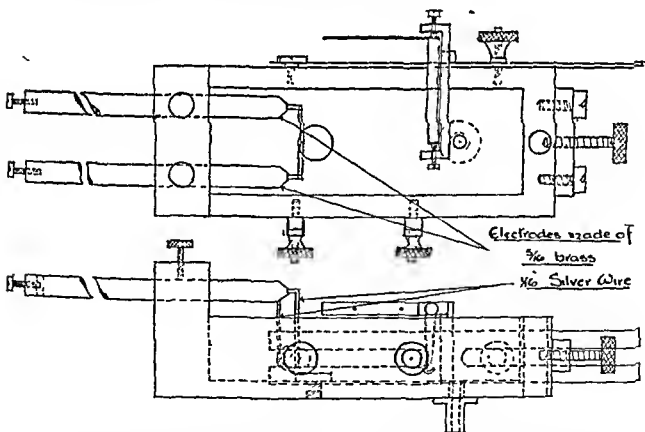
It will be seen that in each set of experiments the dog receiving the thyroid gland by mouth has developed the worse rickets. The potency of the thyroid in stimulating the metabolism is evident in the smaller increase in weight of animals receiving this substance.

A further point of interest is that the rickets-producing effect of the thyroid appears to diminish with advancing age. In general, puppies receiving thyroid show in course of time greater curative changes than those usually seen in dogs with the same amount of rickets when there is no alteration of diet or environment.

### A Keith Lucas Muscle Trough with modified electrodes.

By A. C. DOWNING.

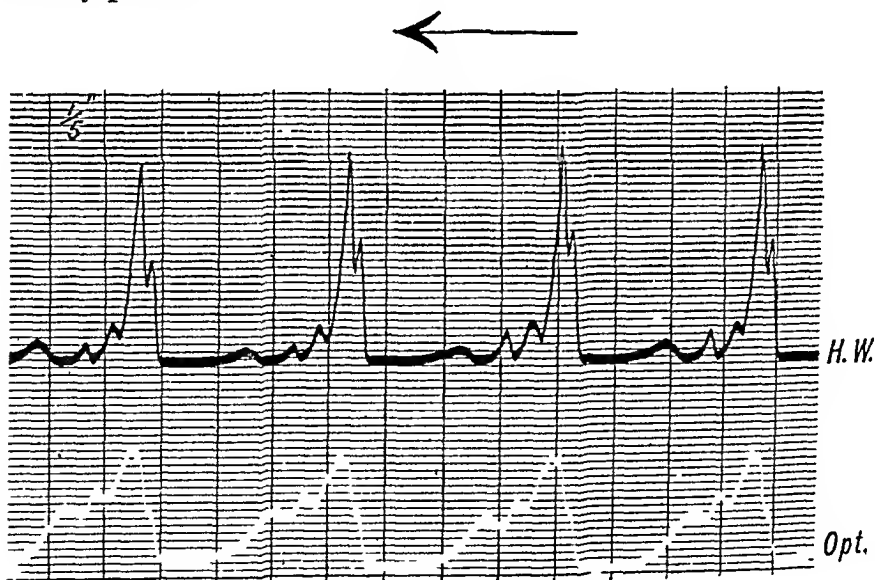
The electrodes of the usual Lucas muscle trough are apt to break, are difficult to mend, and allow the solution to escape along the tubes holding them. A modified trough has been made in which the electrodes consist of brass rods, held in holes above the level of the solution, in an elevation of the wall as in the figure, and furnished with silver-wire terminations for applying to the muscle or nerve. The silver wires may be used either as non-polarisable AgCl electrodes, or as ordinary polarisable ones, and they can immediately be restored if broken off.



The trough has been simplified by the omission of the second set of electrode holes, and the second set of terminals, and is appreciably simpler to manufacture than the older type. The terminals are used merely to hold the wires running from the stimulating coil to the electrodes, and not to make an electrical contact with them.

**Hot wire record of rapid pressure vibrations in the carotid artery.** By J. CRIGHTON BRAMWELL\* and B. A. MCSWINEY.

In certain carotid records from cases of aortic incompetence the first wave of the hot wire sphygmogram instead of being represented by a simple deflection exhibits two or three peaks. These secondary peaks appear to be caused by vibrations set up in the artery by the initial shock due to the sudden discharge of an abnormally large volume of blood from a dilated and hypertrophied left ventricle into an arterial system in which the diastolic pressure is unduly low. They appear to be of the same nature as the notches on the upstroke of the radial sphygmogram described by Feil and Gilder(1). Similar systolic vibrations in the optical pressure records in the central arteries have been recorded in normal animals by Wiggers(2) and other observers. This abnormality of the hot wire sphygmogram does not appear to be confined to cases of aortic incompetence, and has been recorded by us in certain other pathological conditions. We are, therefore, inclined to think that it is attributable not to the presence of any additional factor in these conditions but rather to an amplification of the vibrations which are normally present.



Synchronous hot wire and optical sphygmograms.

<sup>1</sup> Feil and Gilder, *Heart*, 8. 1921.

<sup>2</sup> Wiggers, *Amer. J. Physiol.* 56. 1921.

\* Working for the Medical Research Council.

**An easy method of recording venous pressure.**

By R. J. S. McDOWALL.

The method consists essentially of connecting the vein to a bicarbonate manometer recording directly on the drum by means of a hollow vulcanite float carrying a writer. The cannula used is of the ordinary arterial type, but of larger bore and of sufficient length to pass into the thorax or abdomen according to whether the femoral or the external jugular is used. For prolonged experiments the length is essential as with shorter cannulae the vein is liable to be kinked, while a minimum of dissection is carried out that the vein may be maintained in its natural alignment by its own attachments. Special attention is paid to the heveling of the cannula in order that the opening be not directed against the wall of the vein.

The manometer may be a straight tube such as a narrow burette, if it is convenient to have the animal on approximately the same level as the drum of the kymograph. A three-quarters saturated sodium bicarbonate is used to prevent clotting and does no serious harm if a little gets into the animal.

The float is bell-shaped, and may be made from a small fountain-pen cap made thin and air-tight. This will easily carry a light aluminium writer.

Pressures from  $-10$  to  $100$  may be recorded easily and no serious difficulty is experienced in experiments lasting several hours.

The method obviates the necessity of piston or bellows recorders which are liable to leak.

**The refractory period of nerve as measured by the electrical change in muscle.** By B. A. McSWINEY and S. L. MUCKLOW.

The refractory period of a nerve fibre is usually measured by recording the mechanical response in an attached muscle to two successive stimuli(1, 2). Gotch and Burch(3, 4) have shown that the refractory period can also be measured by recording the electrical change resulting from two such stimuli.

We have measured the refractory period by recording the electrical response to two stimuli by means of a moving coil galvanometer(5) which has the advantage that the deflection of the galvanometer can be read off the scale without making photographic records. The experiments were made on the sciatic gastrocnemius preparation of the

frog by the usual method. If there is a very short interval between the shocks given by the opening of two keys of a Keith Lucas contact breaker, the total electrical change is the same as that produced by one maximal shock. As the interval increases the deflection of the galvanometer becomes larger. The point at which the galvanometer reading commences to increase is taken as the refractory period.

In frog muscles at 12° C. the refractory period of a nerve fibre in a number of experiments was found to vary between .002 and .0025 of a second. These results agree with those obtained by other observers.

The expenses of this investigation were defrayed by a grant from the Royal Society.

<sup>1</sup> Bramwell and Keith Lucas, *Journ. of Physiol.* 42. p. 495, 1911.

<sup>2</sup> Adrian and Keith Lucas, *ibid.* 44. p. 68, 1912.

<sup>3</sup> Gotch and Burch, *ibid.* 24. p. 410, 1899.

<sup>4</sup> Gotch, *ibid.* 40. p. 267, 1910.

<sup>5</sup> McSwiney and Mucklow, *ibid.* 56. p. 397, 1922.

**Effect of rise of blood pressure on the form of the electrocardiogram.** By G. N. LANGLEY, B. A. MCSWINEY, S. L. MUCKLOW, J. B. STOPFORD and S. R. WILSON.

These experiments were done to show what change, if any, could be demonstrated in the form of the electrocardiogram as the result of suddenly raising the blood pressure to a considerable degree.

The methods adopted were (1) to clamp the aorta immediately above the diaphragm. The blood pressure immediately rose by 29 mm. Hg. and on relaxation of the clamping fell again to normal. The form of the electrocardiogram before, during, and after showed no change; (2) the intrathoracic pressure was raised and maintained for a short period by insufflation. The blood pressure fell by 48 mm. Hg. and remained down; on relaxation the pressure immediately returned to normal. Electrocardiograms before, during, and after insufflation proved to be identical.

These experiments were performed as preliminary to investigation of the effect of intravenous injection of adrenalin; and to whatever cause the profound electrocardiographic changes may be due, it seems clear they do not result from pressure changes alone.

This work is proceeding and it is hoped in the future to make a further communication.

The expenses of this investigation were defrayed by a grant from the Royal Society.

**Indirect electrocardiographic leads applicable to children and experimental animals.** By G. N. LANGLEY, B. A. McSWINEY, S. L. MUCKLOW, J. B. STOPFORD and S. R. WILSON.

These were devised so as to be equally useful in any position of the electrodes and have chiefly been used by us for Lead 2 electrocardiograms in animals under anæsthesia.

The limbs are thoroughly rubbed with 5 p.c. salt solution and then enveloped in gamgee tissue soaked in the same. The enveloped limb is now packed into a porous pot which in turn is wrapped with more gamgee tissue soaked in zinc sulphate solution and including a zinc rod as used in a Leclanché cell. The whole is now contained in an earthenware jar.

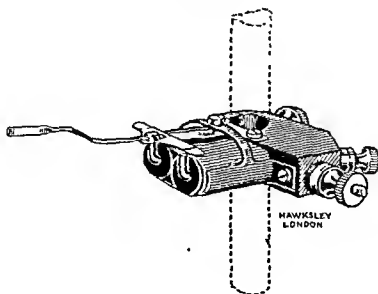
The gamgee tissue should not be sufficiently wet to leak when the pot is laid on its side.

These electrodes are completely non-polarisable and can be used in any position found convenient, the only necessary precaution being to prevent their rolling.

They have been found equally convenient for taking clinical electrocardiograms from very young children.

**A new time signal.** By R. J. S. McDOWALL.

The signal was specially designed to permit of several being placed one above the other with a minimum of space intervening. It is on the principle of some of the large commercial magnets. The cores are prolonged beyond the coils and bent as required. It is made by Hawksley and Sons. The drawing shows actual size.

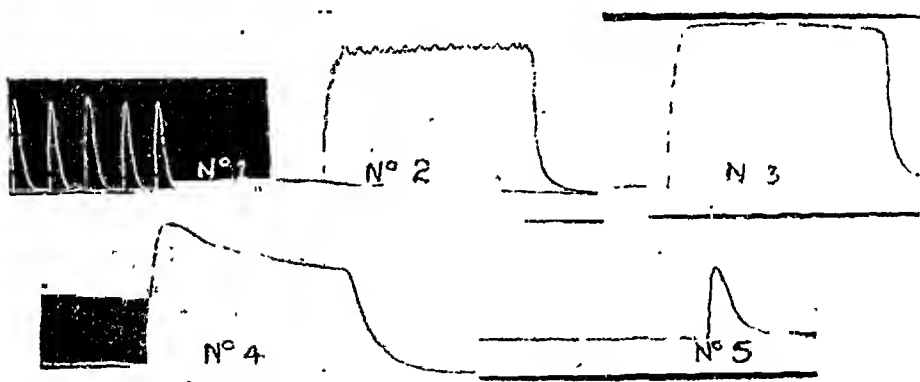




**A small A.C. dynamo for variable frequency stimulation.**

By A. C. DOWNING.

A small A.C. dynamo (part of a pedal-cycle lighting set) was fitted with a  $\frac{1}{2}$ -inch pulley grooved out to take a rubber band. Mounted on a rigid stand the dynamo—which has ball-bearings and is perfectly balanced—was driven by the band being brought in contact with a revolving flywheel, the ratio of speeds being 9:1. Friction between band and flywheel was sufficient to allow the attainment of any desired velocity, without the troubles attending the use of a belt at high rotational speeds.



No. 1, 1.5 stimuli per sec.: No. 2, 6 per sec.: No. 3, 78 per sec.: No. 4, 1200 per sec.: No. 5, 1542 per sec. No. 5 shows an initial twitch followed by complete inhibition.

The dynamo develops about 4 volts and about 5 watts at (and above) 3000 r.p.m.: at low speeds the E.M.F. and output are less, but sufficient to stimulate a nerve down to quite low frequencies (e.g.  $1\frac{1}{2}$  times per sec.). There are three complete cycles, i.e. six shocks, per revolution, and it is possible to stimulate a nerve over the complete range 1.5 to 2000 shocks per sec. If required a small transformer may be used at all but the lowest speeds to increase the E.M.F.

In the figure five different frequencies of stimulation are shown: the highest but one shows a partial oncoming inhibition, and the highest a complete inhibition—following the first twitch—resulting from a prolonged stimulus.

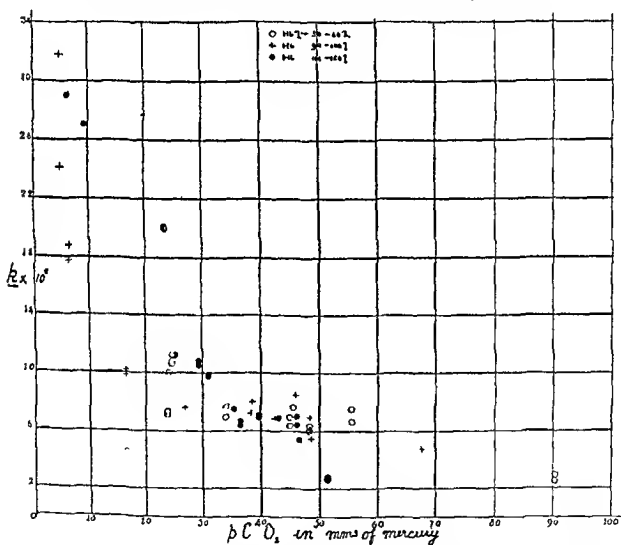
**The constancy of the ratio  $c.H$  to  $p.CO_2/v.CO_2$  in blood of varying hæmoglobin content.** By RUTH E. CONWAY and DORIS M. THOMAS.

A series of nearly fifty experiments have been made on blood in order to ascertain whether  $k$  in the equation  $c.H / \frac{p.CO_2}{v.CO_2} = k$  is constant for varying percentages of hæmoglobin present in the blood, and for varying  $p.CO_2$ . Here

$c.H$  represents the  $H$  ion concentration,

$p.CO_2$  the pressure of carbon dioxide in mm., and

$v.CO_2$  the volume of combined  $CO_2$ , in c. e. per cent.



Defibrinated ox blood of known hæmoglobin percentage was treated with 0.5% sodium fluoride and exposed for at least two hours at 15° C. to a known pressure of  $CO_2$ . The latter was estimated by the Haldane gas analysis apparatus, in a sample of gas taken from the tonometer after equilibration with the blood, and corrected to represent the pressure which the same concentration of gas would have exerted at 0° C. The

values of  $c.H$  were obtained by the method of "dialysis" at room temperature, and corrected to represent the  $c.H$  at  $15^{\circ}\text{C}$ ., using the correction suggested by Cullen, which in effect means subtracting  $2.3\%$  from the observed  $c.H$  for every  $1^{\circ}\text{C}$ . above  $15^{\circ}\text{C}$ . The volume of combined  $\text{CO}_2$  was obtained as follows. The total  $\text{CO}_2$  contained in the blood was observed by means of the Haldane blood gas analysis apparatus, and reduced to N.T.P. The  $\text{CO}_2$  dissolved in the blood was subtracted: the latter was obtained by applying Bohr's factor, and employing a temperature coefficient of solubility the same as in the case of water.

By these methods  $k$  does not appear to be a constant figure as  $p.\text{CO}_2$  varies, but decreases as  $p.\text{CO}_2$  rises. By plotting  $k$  against  $p.\text{CO}_2$  it will be seen from the figure that

- i. blood anæmic within the range of  $50\text{--}60\%$  Hb,
- ii. normal blood, i.e.  $90\text{--}100\%$  Hb,
- iii. blood rich to excess in Hb,  $114\text{--}150\%$  Hb,

are indistinguishable from one another, the readings especially within the range of  $30\text{--}55$  mms. pressure of  $\text{CO}_2$  being mixed indiscriminately with one another. Within this range  $k$  is a reasonably constant figure, but readings above and below these limits of  $p.\text{CO}_2$  indicate very definitely a variation of  $k$  with  $p.\text{CO}_2$ .





# PROCEEDINGS

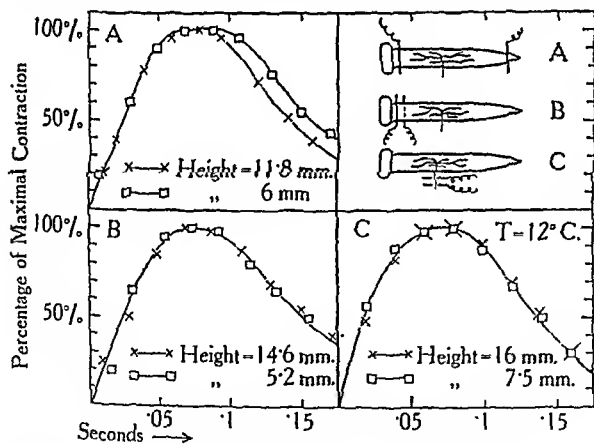
## OF THE

# PHYSIOLOGICAL SOCIETY,

*December 16, 1922.*

**The time relations of the isometric twitch.** By E. D. ADRIAN.

Investigating the isometric twitch of the frog's sartorius Hartree and Hill(1) find that an alteration in the strength of the stimulus causes a change not only in the maximum tension developed, but also in the time relations of the twitch. With a strong stimulus they find that the muscle relaxes more rapidly than with a weak, and they point out the



Frog's sartorius. Time relations of isometric twitches due to strong and weak stimuli with different arrangements of electrodes.

difficulty of explaining this if each fibre contracts on an all-or-nothing basis. A possible explanation is supplied by the fact that their stimulating electrodes were placed one at the tibial and one at the pelvic end of the muscle. With this arrangement a weak stimulus would take effect at

the kathode at one end of the muscle, and the wave would travel its entire length before the twitch came to an end. A strong stimulus might take effect on the nerve endings and nerve fibres in the middle region of the muscle and the wave of contraction would then spread out in both directions, so that the twitch would be over in a shorter time. A corresponding shortening of the electric response was found by Adrian and Owen<sup>(2)</sup> when the sartorius was stimulated by way of its nerve instead of by electrodes at one end.

I have tested this suggestion by photographing the isometric twitch of a sartorius muscle with an optical lever magnifying about 400 and having a periodicity of about 250 a second. The muscle was stimulated with break shocks of different strength by electrodes placed (a) at either end of the muscle, (b) both at the pelvic end and (c) both on the nerve. If the suggestion is correct we should find the change of form present with arrangement (a) where the point of incidence of the stimulus can shift from muscle to nerve, but absent in (b) or (c). The figure shows three sets of curves from one muscle and it will be seen that the effect is present only with arrangement (a). The same effect was present in eight out of nine experiments with electrodes arranged as in (a), with (c) there was no sign of it, with (d) a small change was present in two experiments. In the figure with electrodes at either end of the muscle the slow curve shows a lag of  $\cdot 015$ – $\cdot 02$  sec. over the rapid; this implies a slow rate of conduction in the muscle, but one which agrees well with the difference in the time relations of the electric response shown by Adrian and Owen.

These results suggest that the change in the form of the twitch depends on an arrangement of electrodes which allows the point of stimulation to alter as the strength is increased. If this is so, the effect is quite compatible with an all-or-nothing response at each point in the muscle-fibre.

In more complex muscles some other cause seems to produce changes in the form of the twitch according to the number of fibres in action. In the gastrocnemius stimulated via the nerve the submaximal contraction is sometimes longer and sometimes shorter than the maximal. The change seems to depend on the number of fibres in action and not on the strength with which they are stimulated, for it occurs when the submaximal contraction is produced by a maximal stimulus limited to one nerve root.

(1) Hartree and Hill. *Journ. of Physiol.* 55. p. 403. 1921.

(2) Adrian and Owen. *Ibid.* 55. p. 329. 1921.

**On an enzyme responsible for alteration of the rotatory powers of glucose and fructose. By L. B. WINTER and W. SMITH.**

In a paper shortly to be published we show that the normal blood sugar in man and animals has a lower rotatory power than would be given by the  $\alpha$ - $\beta$  equilibrium form of glucose as deduced from the copper reducing power. We also show that this sugar is almost, or entirely, absent from the blood of persons suffering from diabetes mellitus; and it is suggested that in normal subjects an enzyme is responsible for the conversion of  $\alpha$ - $\beta$  glucose into normal blood sugar; the absence, or inactivation of this enzyme is suggested as the direct cause of the disease.

We have examined tissue extracts to determine the seat of formation and conditions of activity of the enzyme. The liver would appear to contain the enzyme. We have confirmed the observations of Hewitt and Pryde(1) that extracts of the intestinal mucous membrane are inactive in this respect. The enzyme appears to be almost inactive in the absence of an extract of the pancreas.

The extracts of pancreas which we have recently used are preparations of 'Insulin' obtained by Collip's method(2). Similar activation of the enzyme is obtained.

Solutions of glucose or fructose when incubated at 37° C. with very small amounts of insulin and liver extract in a jacketed polarimeter tube have their rotations altered in a downward and upward direction respectively. In view of the fact that our insulin preparations invariably contain phosphate, it is noteworthy that addition of phosphate accelerates the reaction to a degree not obtained by the use of other salts. Phosphate buffers have therefore been employed. Boiled liver extract is inactive, whereas insulin would appear to be thermo-stable in this respect. Our insulin preparations were tested for activity on rabbits.

The copper reducing power of the sugar solution at the beginning and end of the reaction is unaltered. The reaction is therefore different from that described by Levene and Mayer(3) in connection with muscle and pancreas extracts, in which the copper reducing power was lowered.

REFERENCES.

- (1) Hewitt and Pryde. *Biochem. Journ.* 14. p. 395. 1920.
- (2) Collip. *Proc. Roy. Soc. Canada.* 1922.
- (3) Levene and Mayer. *Journ. Biol. Chem.* 9. p. 97. 1911.



**The automatic integrating katathermometer.**

By E. H. J. SCHUSTER and H. C. SAYER.

This instrument is a modification of Leonard Hill's katathermometer. Its objects are to heat the bulb (*K*) of the katathermometer to  $100^{\circ}$  F., to allow it to cool to  $95^{\circ}$ ; then immediately to re-heat it to  $100^{\circ}$ , again to allow it to cool to  $95^{\circ}$ , then to re-heat it and so on indefinitely; also to record the total length of time in seconds during which it has been cooling and the number of times which it has been allowed to cool. The total cooling time divided by the number of cooling periods between two successive readings of the instrument gives the average number of seconds in one cooling period. If the number of seconds thus arrived at

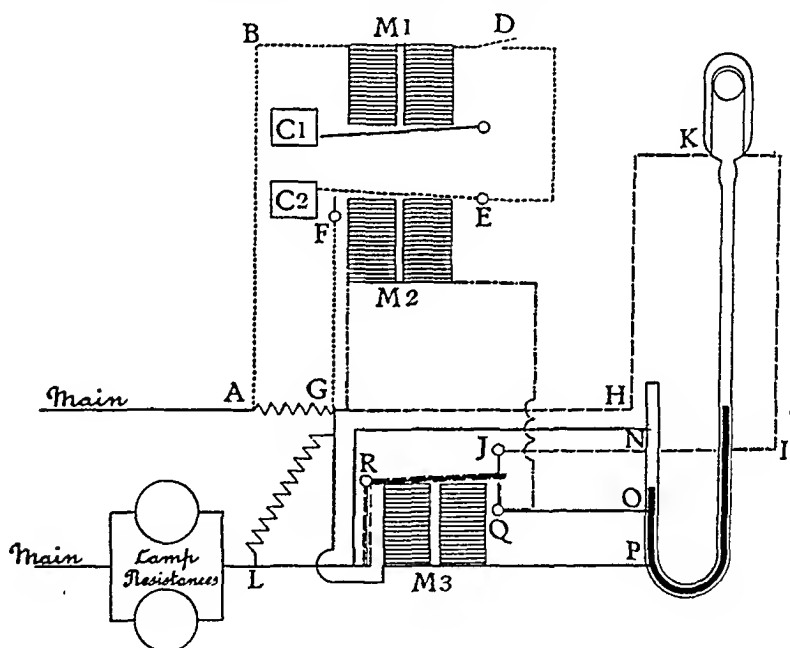


Diagram of Circuits.

is divided into a factor determined for the particular instrument, the cooling rate in millicalories per square centimetre of bulb surface is obtained; this is the measure of the cooling power of the air in the units usually employed for katathermometer cooling powers.

The liquid in the bulb of the instrument is toluol; below the toluol is a mercury seal which extends round the bend of the capillary tube and up into its shorter limb. Platinum wires are fused into the shorter limb at points *P*, *O* and *N*. The mercury is at point *O* at  $95^{\circ}$  F., and at point *N* at  $100^{\circ}$  F. The diagram represents the electrical connexions

when the mercury is rising from *O* to *N*. Current flows between the mains by the circuit *A, G, H, K, I, J, R, L*. In the bulb *K* it passes through a loop of resistance wire from which the necessary heat is conducted into the toluol. While the current is flowing through this path the mercury continues to rise until it reaches the point *N*, when the circuit *A, G, M3, P, N, L* is closed owing to the mercury coming into contact with the platinum wire at *N*.

At *M3* is an electromagnet, which, when the current passes through it, attracts its armature and thus breaks the connexion *R, J*. The heating of the bulb therefore immediately stops and the mercury starts to fall again. At the same time the connexion *R, Q* is established which is a link in the circuit *A, G, M3, P, O, Q, R, L*. This maintains the current through the magnet *M3* and thus prevents the connexion at *J* being re-established until the mercury has fallen below the point *O*. When it has fallen to that level, the circuit through the magnet is broken and the heating circuit is re-made and the mercury starts to rise again, repeating the same cycle of operations indefinitely.

The recording mechanism is operated in the following manner: When connexion is established between *Q* and *R*, the current flows through the magnet *M2* by the path *A, G, M2, Q, R, L*. The armature lever therefore moves towards the magnet. A pin at the end of it works in a slot in the arm of the Veeda counter *C2*. Thus by the movement of the lever an additional unit is registered on the counter. As the lever moves towards the magnet at the beginning of each cooling period and away from it at the end of the period, the counter *C2* registers the number of periods.

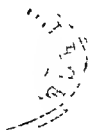
A further effect of the movement of the lever is to establish a connexion between *E* and *F* which is a link in the magnet circuit *A, B, M1, D, E, F, G*. At point *D* connexion is made or broken every second. In the experimental apparatus exhibited, a metronome was used for the purpose. Thus when the connexion *E, F* is made, the armature lever of the magnet *M1* moves backwards and forwards once every second. It works the Veeda counter *C1*. As the connexion *E, F* is only made while the katathermometer is cooling, the counter *C1* registers the number of seconds of cooling time.

It should be mentioned in conclusion that the proper distribution of current in the various circuits is effected by adjusting the lamp resistances, the resistances of the magnets themselves and the shunt resistances between points *A* and *G* and *G* and *L*. The instrument could be readily adapted to work from accumulators.



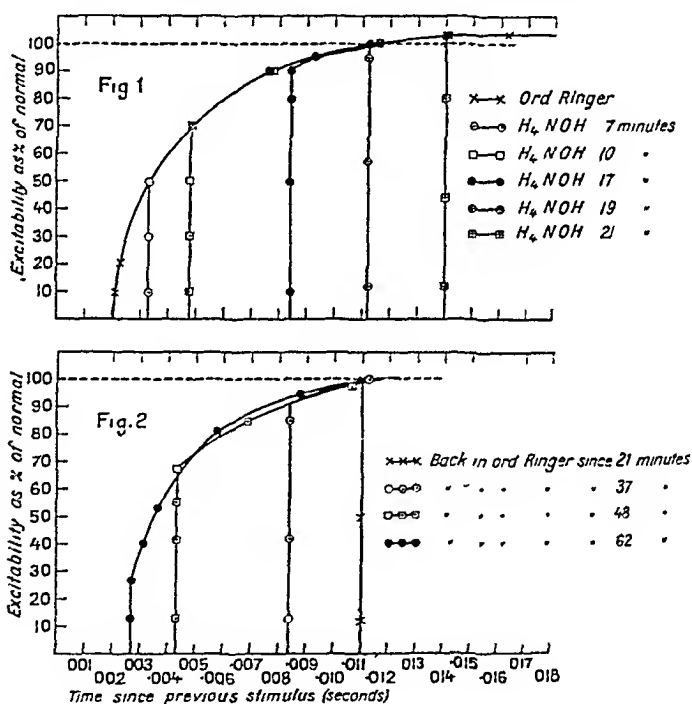
that the friction of the drum against the point tends to carry  $DF$  into the hinge (the arrow in Fig. 1 shows the direction of the movement of the drum). The pen can be used for lateral writing when it can be made very much smaller, the hinge and carrying arm can be discarded and the pen attached to a flexible mount such as the Bayliss hinge. For the dimensions of the pen given in Fig. 1 the error of the line inscribed as compared with the arc are described by the hinge  $H$  is less than 1.0 mm. when the lever moves from  $10^\circ$  below the horizontal to  $20^\circ$  above, and when  $H$  is not further than 1 cm. from the drum with the lever horizontal. The same applies to the relationship of the vertical distance through which  $H$  moves. The error is of course the same as for a writing point made on the Lovatt Evans model in which the distance from the hinge to the writing point is the same as the distance  $HA$  in this pen.

The nearer the hinge  $H$  is to the drum with any form of frontal lever the more accurately the line inscribed on the drum represents the arc described by the hinge, as can be shown by quite simple graphic methods.



produces a decrement in conduction which increases progressively. The determination of the recovery curve is clearly a valuable method of testing the effect of any agent on the conductivity of the nerve ending. The success of two stimuli after one has become ineffective is no doubt due to the supernormal phase of recovery. A second impulse occurring 0.011 sec. after the first would be of greater size and so would stand less chance of extinction.

The curare-like action of certain  $\text{NH}_4$  salts has been known for some time (cf. Hofmeister<sup>1</sup>), but it seems to us to depend on a special action of  $\text{NH}_4\text{OH}$  or  $\text{NH}_4$  ions rather than on its  $\text{CO}_2$ -binding action, since we are unable to confirm Mansfeld's results with  $\text{NaOH}$  and  $\text{Na}_2\text{CO}_3$ .



### The inception of the coagulation of human blood.

By J. W. PICKERING.

The appearance of filaments in human blood within ten seconds of its issue from a wound was described by Buckmaster<sup>(1)</sup>. Addis<sup>(2)</sup> confirmed these observations; but did not regard the threads as examples of coagulation, a considerable time having elapsed between their forma-

<sup>1</sup> Hofmeister. *Arch. f. exp. Path. u. Pharm.* 16. 1893.

tion and that of a typical clot. From experiments on cataphoresis, Howell(3) distinguished two stages in the clotting of fibrinogen by thrombin, first the formation of a delicate membrane, later the appearance of a gelatinous clot. Pickering and Hewitt(4) found reversible gels, prior to clotting, in blood shed under oil.

The writer's blood was obtained from punctures in the skin. The room temperature was 17° to 18° and the air was kept humid by jets of steam. Under these conditions, filaments were formed on the surface of the blood exposed to air, within 15 or 20 seconds after the bleeding. When these threads were immediately removed by a needle coated with paraffin wax and at once placed on a paraffined slide they were found to dissolve in tap water at room temperatures. When the filaments were removed by an uncoated steel or glass needle they did not dissolve in tap water. Threads remaining in the blood for 50 seconds, or longer, after detection by passing a paraffined needle through the blood, either failed to dissolve, partially dissolved or dissolved with difficulty. The first alternative was the more common. Failure to obtain solution of the threads usually occurred when the blood was shed in a room with a relatively dry atmosphere. The threads found in blood obtained by again pricking a puncture which had stopped bleeding were insoluble. These observations indicate that the commencement of coagulation may occur earlier than is commonly recognised and point to the initial phase of that process being reversible, even when the blood has, for a short time, been in contact with air and damaged tissues. It would also appear probable that what are commonly described as the pre-clot changes in blood are concomitant with the formation of films or filaments. It is difficult to regard the phenomena described as due to the rapid formation of thrombin, it being known that blood immediately shed into alcohol does not yield that coagulant. The observations of Ramsden(5) may be recalled that, apart from the effects of evaporation, solid or highly viscous coatings are more or less rapidly formed on the free surface of all protein solutions. Thus is a physical interpretation of the inception of coagulation indicated.

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period, some of the corpuscles appeared swollen and spherical but the majority were apparently normal. The gum-saline thus protects erythrocytes from the action of X-rays. Control experiments showed that in the absence of irradiation hæmolysis was absent, a few crenated corpuscles were found in the suspensions in either Locke-Ringer solution or in normal saline but crenation was absent when isotonic gum-saline was used. The addition of .25 p.c. of sodium citrate to either the irradiated suspensions or to those not irradiated did not modify the results. The gum acacia used contained ionised calcium and experiments are in progress to ascertain whether the protective action of gum-saline may be assigned to the presence of calcium salts.

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#### **Some new aspects of the function of the skin in temperature regulation.** By W. A. OSBORNE.

I have shown (*Journ. Phys.* 41, p. 345. 1911) that the skin is to be regarded as a gel containing water of imbibition. Evaporation of this water can occur independently of that of sweat and, when the air is cold, dry and in rapid movement, such evaporation of water may be contrary to the metabolic requirements of the body and may alter the texture of the skin. Such actions are probably the cause of the discomfort produced by the east wind in N.W. Europe.

*Concentration of Salts on the Skin's Surface.* It is well known that after immersion in water the outer epidermis swells and becomes furrowed. In this state the skin gel has imbibed fluid and has expanded. If the hands are held in isotonic salt solution a similar change occurs. This is not due to the absence of protein from the salt solution for, if the hands are held in ox blood, swelling and furrowing of the skin also occur. The change in the skin can only be prevented by greatly increasing the osmotic pressure of the salt solution. In the experiments I have made it was not till the solution contained 10 p.c. of sodium chloride that the skin remained normal after being immersed for an hour. Now sweat is a hypotonic fluid and might be thought to produce swelling of the epidermis but, owing to surface evaporation, there is considerable concentration. Indeed in some climates it is surprising that there is not crystallisation on the skin surface. The absence of such may be due to the presence of small amounts of calcium salts or to organic matter.

*Cold and Moist Air.* The uncomfortable feeling caused by cold moist air is attributed, so far as any explanation has been given, to the moist air being a better conductor of heat than dry air. This cannot, however, be the correct explanation, for the diathermic properties of all ordinary gases and mixtures of gases are very close to each other. The cause I take to be that in moist air there is a partial equilibration of the skin gel with the vapour pressure of water in the air leading to a swelling and to a diminution of the small air spaces. The swelling is, in fact, detectable with a strong lens or with a low power of the microscope. As a result of this diminished air enclosed between the epidermic scales the conducting power of the skin is increased.

*Oiling the Skin.* Oiling the skin is a common practice in tropical and sub-tropical climates. It is held to be protective when the wind is hot and dry. No satisfactory explanation of its protective power has been advanced. I have not found that it has any material action on the rate of evaporation. Possibly its effect may be due to the oil filling up the air spaces between the epidermic cells and so increasing conduction when the air is below body temperature.

**Some observations on colour blindness. (*Preliminary Communication.*) By H. E. ROAF.**

In order to study colour vision the following method has been used. An outline diagram is coloured by water colour paints, and an individual known to be colour blind is furnished with a similar diagram and the same paints. The subject is told to test the colours on a spare piece of paper and to fill in the diagram to correspond to the coloured one supplied. It was expected that if the colour blind individual failed to recognise any region of the spectrum he might omit that region in some matches and add it in others where it is absent in the original.

The original and copy are compared, using colour screens until the two appear to be matched. By spectroscopic examination of the colour screen the amount of spectrum cut off can be determined.

By this preliminary method seven examples<sup>1</sup> have been studied and in all of these the diagrams matched after cutting off more or less of the red of the spectrum whilst cutting off all but the red accentuated the differences. It must be remembered, however, that the individual cannot be said to be blind to the red of the spectrum but only that he fails to

<sup>1</sup> I am indebted to Capt. J. H. Shaxby, of University College, Cardiff, for three of these examples.



differentiate it from other colours. Actual blindness to that region would cause shortening of the spectrum, which is not usually the case.

Examination by colour screens does not give sufficiently accurate information as to the region of the spectrum affected, so a colour lantern of the type shown before this society by Prof. A. W. Porter, F.R.S., is being assembled. With it it may be possible to discover the region and extent of the spectrum, removal of which allows the diagrams to be matched.

The results obtained up to the present suggest that, with impure colours the eye performs a spectrum analysis and that the colour blind individual fails to recognise the full effect of a portion of the spectrum unless aided by other factors such as texture or shade.

I am publishing this communication in the hope that I may hear of further cases of colour blindness for similar study.

#### **A simple myocardiograph. By O. INCHLEY.**

This consists of a receiver and recorder connected by tubing. The receiver (Fig. 1) is a small drum, the opposite drumheads being pressed upon by levers attached to the heart. The body of the drum is made of aluminium sheet bent in the form of a hoop; it is one cm. in depth and four cms. in diameter. The ends of the strip are held between two shaped pieces of cork which are pierced by a short length of glass tube. These are all tied together with string or fine wire and sharp points are smoothed away or covered with adhesive strapping and French chalk applied. The whole is now inserted into a condome. The membrane is adjusted to leave the tambour areas smooth so that the folds occur over the metal hoop: the membrane is fixed in position by a few turns of string round the outer cork, and tied round the glass tube above. The levers are carried by another cork impaled by the glass tube; this cork has two small glass tubes tied to it, through which the aluminium wire levers (gauge about 19 B.W.G.) are passed and shaped as in the figure. The wires pass through cork tambour-pieces two cms. in diameter at a distance of half a cm. from the membrane. The cork should project so as to be easily held securely by the fingers when bending the lever-ends for adjustment to the heart. The wires are lashed together below with fine brass wire, coated with Prout's glue to which a needle, about No. 9 size, is fixed. The receiver is supported by rubber tube in the manner shown in the figure, thus allowing freedom of movement. Adjustment of the receiver is made by bending the wire levers till the points are

at the distance to give the necessary tension. The membranes should be under slight tension.

The recorder (Fig. 2) is made by passing a glass tube about five millimetres internal diameter eccentrically through a rubber stopper four cms. in diameter and one and a half cms. thick. A condom is

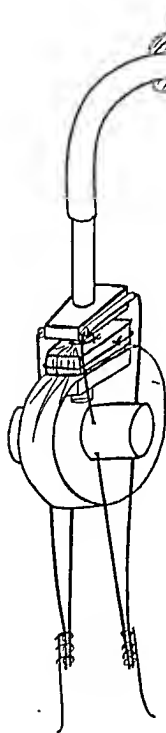


Fig. 1.

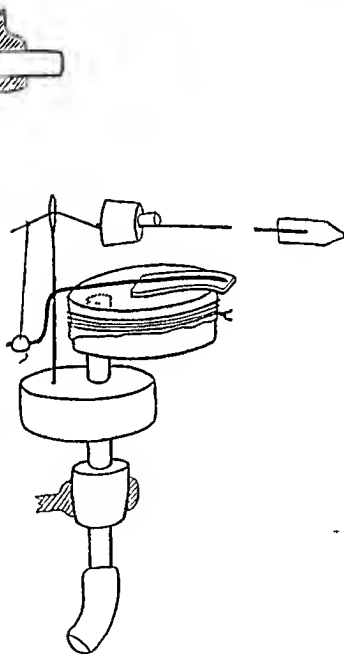


Fig. 2.

applied to the upper surface without stretching and tied on the stopper. On the membrane a strip of canvas-backed tyre patch material, about two and a half cms. long and one cm. wide, is cemented from the center to well over the edge. On to this is glued a piece of aluminium

shown in the figure. Below the rubber stopper the glass tube carries a cork which bears a vertical darning needle, No. 4 size. Through the eye of this a fine sewing needle, bent in the flame, is passed. To this a fine silk thread is tied, the free end of which is wrapped about the end of the aluminium wire and held by plasticine. The point of the needle carries a small cork which serves to hold the light lever. The apparatus is supported below. The lever is adjusted by sliding the cork carrying the darning needle. The membrane, being applied without tension, is stretched upward by the weight of the lever, so forming an air-space beneath.

The instrument, calibrated by calipers, shows that under ordinary tension movements of the receiver produce uniform movements in the recorder.

# PROCEEDINGS

## OF THE

# PHYSIOLOGICAL SOCIETY,

*February 17, 1923.*

Some evidence for the existence of polysaccharides in the blood of diabetics. By L. B. WINTER and W. SMITH.

The results of previous observations on diabetic blood, in which the polarimetric value was greater than the copper reduction value reckoned as  $\alpha$ - $\beta$  glucose, suggested that besides  $\alpha$ - $\beta$  glucose and a trace of  $\gamma$  glucose, disaccharides might be present.

We have since investigated further cases of diabetes mellitus. We have confirmed the fact that in every case examined the ratio polarimeter to copper reduction value is higher than that given by  $\alpha$ - $\beta$  glucose. The final sugar solution was subjected to acid hydrolysis, each sample was divided into two portions, the one was boiled with dilute acid for a short time, the other was boiled with stronger acid for a considerable period. In the first case there was always a large rise in the dextro-rotatory powers of the solution with little change in the copper reducing power, whilst in the second case, the copper value and polarimeter were as for  $\alpha$ - $\beta$  glucose in equilibrium.

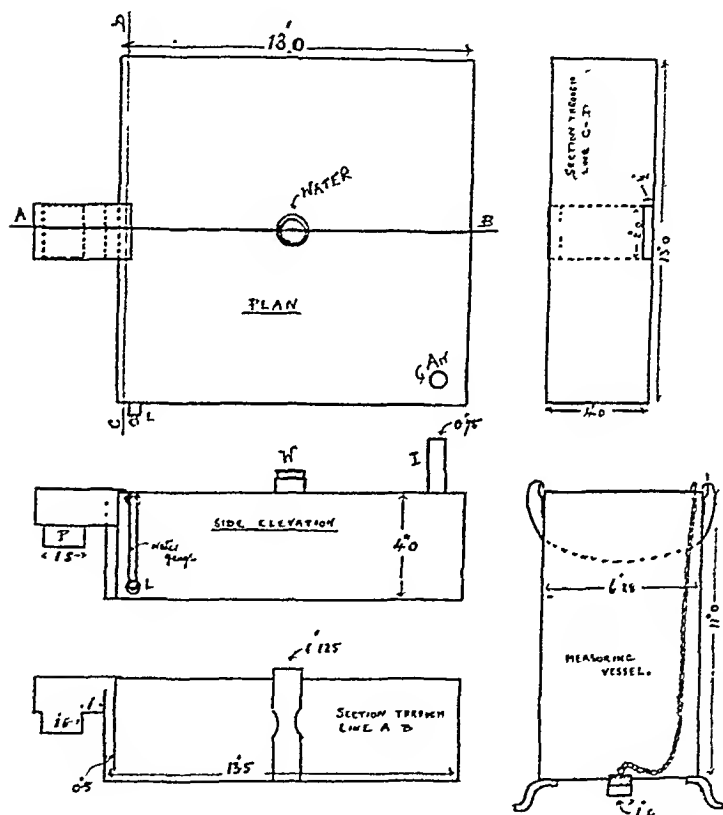
We suggest that in diabetic blood, besides varying amounts of  $\alpha$ - $\beta$  and  $\gamma$  glucose, there are complex sugars which are readily hydrolysed into simpler components, but which require prolonged treatment before they are resolved into  $\alpha$ - $\beta$  glucose. We have found that in some experiments on which we are at present engaged hydrolysis of the blood sugar of animals under certain conditions gives results comparable to those obtained from the blood of diabetic persons.

After injection of insulin the blood sugars of diabetic persons undergo a considerable change in their relative proportions; hydrolysis of the final product for a short period results in little if any increase in the polarimeter to copper ratio. The case which showed the least change was that which appeared to have benefited most from the insulin, as evidenced by the fact that the initial polarimetric reading before hydrolysis was below that of the copper reducing value calculated as  $\alpha$ - $\beta$  glucose.

**A wet spirometer.** By J. G. WOOLHAM.

The wet spirometer consists of a metallic box which has two inlet pipes, one for air (*I*) which is blown into the spirometer to be measured, the other (*W*) for water which is run into the box to within one half inch of the top; the box has one outlet pipe (*P*) for the water which is displaced by the air which is blown into the box.

The box measures 13" by 13", top and bottom, and it is 4" deep.



The air inlet pipe (*I*) is situated at one of the corners of the top of the box, it stands erect to a height of 2", it has  $\frac{3}{4}$ " bore.

The water inlet pipe (*W*) is placed at the centre of the top of the box, the pipe extends to the bottom of the box where it is soldered, and it projects 1" above the top of the box. This water inlet pipe acts as a stay inside the box, it is soldered to the bottom and also where it perforates the top; it is perforated by two large openings in the portion of the pipe inside the spirometer. When in use a tube at the bottom of the measuring vessel (to be described later) fits inside the water inlet

and water is run from the measuring vessel into the box. When air is being blown into the spirometer the water inlet is plugged. The box cannot be filled to the top because the water outlet is placed so as to prevent the water attaining a higher resting level than within  $\frac{1}{2}$ " of the top.

The water, when it is displaced by air, leaves the box by a rectangular orifice, 2" by  $\frac{1}{2}$ ", in the side of the box which is farthest away from the air inlet; one of the longer sides of the rectangular orifice rests upon the edge of the bottom of the box.

The tube which conducts the displaced water is rectangular in section and measures 2" by  $\frac{1}{2}$ ". This tube stands vertically touching the outside of the box with the plane of its upper orifice parallel to the side of the box. The sectional area of this tube is one square inch along all its length, but the upper end opens into a tube of much larger sectional area so as to avoid syphonage. The latter tube is fixed at a right angle to the former, and the outlet of this latter pipe is a short tube opening downwards so that water may be run into the measuring vessel which is placed underneath.

The measuring vessel is cylindrical in shape, its diameter is 6.28" and its length is 11". There is a pipe in the centre of the bottom of the vessel which is small enough to fit inside the water inlet of the spirometer box and after the displaced water is measured in the vessel it is returned into the box. Each millimeter of the length of the measuring cylinder wetted represents 20 c.c. of the water which is nearly the volume of the air which displaced the water.

The pressure of the air inside the spirometer increases with the drop in the level of the water in the gauge ( $L$ ) and a correction of the volume of air, corresponding to the head of water, may be made.

#### **An analysis of the factors underlying the mechanical efficiency of human muscular movement. By H. LUPTON.**

The total energy liberated in the isolated frog's muscle as a result of a stimulus of duration  $x$  secs. (the complete cycle of activity and recovery being considered) was expressed (1) in the form

$$Q = 2.5 W_0 (1 + bx).$$

The first term— $2.5 W_0$ —represents the energy expenditure as the result of the setting-up of a state of potential energy  $W_0$ ; the second term— $2.5 b W_0 x$ —represents the energy used as a result of maintaining a state of potential energy  $W_0$  for a time  $x$  secs. The value of the

The free acid causes epithelial desquamation and other signs of inflammatory reaction.

Triolein absorption is free from irritating effects and the histochemical picture is distinct.

In view of the possibility of the formation of glycerol from glucose, mixtures of oleic acid and glucose and of mannitol olein have been fed to frogs.

The presence of the sugar prevents the inflammatory effect of the oleic acid and delays to some extent the staining of the intracellular fat globules.

### **The relation of pituitary extract (infundibular lobe) to the fall of blood sugar produced by insulin.** By J. H. BURN.

Having previously observed that large doses of pituitary extract were able to inhibit a hyperglycaemia produced in normal rabbits by (1) adrenalin, (2) other anaesthesia, I performed experiments to see whether an injection of pituitary extract in any degree intensified the fall of blood sugar produced by injections of insulin.

The following results were obtained:

1. So far from intensifying the fall produced by insulin, a simultaneous hypodermic injection of pituitary extract either reduces, or abolishes, or replaces by a rise of blood sugar, the fall due to insulin.

2. This effect is apparently due to one or other of the active principles of infundibular extract, as it is not produced by similarly prepared extracts of thyroid, thymus, spleen or brain tissue, nor is it produced by histamine. Moreover, this action of pituitary extract is destroyed by such treatment with normal alkali as destroys the active principles without otherwise altering the extract.

3. The doses of pituitary extract used do not themselves produce more than a slight rise of blood sugar.

These results recall the experimental and clinical evidence of Cushing and his co-workers, that in cases of pituitary insufficiency there is a raised carbohydrate tolerance.

### **A correction of the value for the heat of combustion of glycogen.** By W. K. SLATER.

In considering the mechanism of muscular contraction by means of measurements of the heat evolved in various phases of an isometric contraction, and the chemical investigation of the intra-muscular changes,

it became necessary, in order to correlate the results, that the heats of combustion of glycogen and lactic acid should be accurately known.

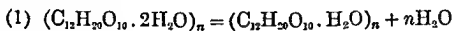
The heat of combustion of lactic acid in the dissolved state has been determined with great care by O. Meyerhof. In the case of glycogen two determinations have been made, one by Emery and Benedict and the other by Stohmann and Schmidt. Both these results give the heat of combustion of solid glycogen in an uncertain condition of hydration.

The present work was undertaken in the first place, to ascertain the order of magnitude of the correction required in the above determinations for heat of solution and wetting.

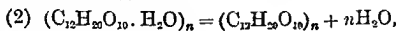
The glycogen was obtained from sea water mussels, and purified by boiling with 30 % caustic potash, and repeated precipitation with alcohol. After the last precipitation the glycogen was freed from alcohol and water, by distilling with anhydrous benzene after the manner described by Atkins. A rapid grinding in a mortar reduced the resulting coarse powder to a fine white amorphous material, which contained only a trace of ash and nitrogen and was free from fat. An ultimate analysis gave  $C = 39.7\%$ , whilst the formula  $(C_{12}H_{20}O_{10} \cdot 2H_2O)_n$  requires  $C = 40.0\%$ .

The amorphous powder was dried over calcium chloride *in vacuo*, and after six days a constant weight was reached with a loss of  $5.02\%$ . The material so dried was placed in an air oven at  $110^\circ C$ . when it immediately commenced to lose more water. After nine days a loss of  $9.6\%$  was recorded without equilibrium having been reached, although it appeared that the loss was slowly approaching a maximum of  $10.0\%$ .

The successive steps in the dehydration can be represented by the following equations:



requiring a loss of  $5.00\%$ .



requiring a total loss of  $10.00\%$ .

The intermediate hydrate  $(C_{12}H_{20}O_{10} \cdot H_2O)_n$  corresponds with the sodium hydroxide compound  $(C_{12}H_{20}O_{10} \cdot NaOH)_n$ , and the completely dehydrated material should be identical with that prepared by Harden and Young, by drying over phosphorus pentoxide *in vacuo* at  $100^\circ C$ .

Preliminary determinations of the heat of wetting and solution of the fully hydrated material, showed this figure to be positive and equal to  $8.8$  cal. per grm., and the sum of the heats of hydration, wetting and solution of the monohydrate to be equal to  $11.8$  cal. per grm.





# PROCEEDINGS

## OF THE

# PHYSIOLOGICAL SOCIETY,

### *March 17, 1923.*

**The fate of some halogen derivatives of benzene and of benzene in the animal body. By T. S. HELE and E. H. CALLOW.**

It has been known since the time of Baumann and Preussc that, when monochlorbenzene is administered to dogs, chlorphenylmercapturic acid and chlorphenylsulphate are excreted in the urine. One molecule of monochlorbenzene corresponds to one atom of sulphur in combination as sulphate or as mercapturic acid. If these are the sole products of metabolism then the sulphur calculated from the output of halogen in organic combination should correspond with the excess sulphur (as ethereal sulphate and as neutral sulphur) over the normal average. The agreement actually is close. This would suggest that there is only one ethereal sulphate and one mercapturic acid present, each containing an aromatic nucleus, to which the halogen is still attached and that in no case is the halogen liberated before either synthesis takes place. The same considerations hold good for some other halogen compounds.

Compound administered	Sulphur calculated from organic halogen gram.	Excess sulphur found experimentally gram.
Monochlorbenzene	0.447	0.407
	0.353	0.352
	1.810	1.870
Ortho-dichlorbenzene	0.362	0.311
	0.348	0.305
	0.454	0.477
Meta-dichlorbenzene	0.454	0.477
Para-bromanisole	0.471	0.462

It is also found that there is a rise of neutral sulphur in the urine of dogs after giving them ortho- or meta-dichlorbenzene, but not after giving para-bromanisole. The rise in neutral sulphur suggests the presence of a mercapturic acid. The failure of the para-bromanisole to produce such an effect may be due either to the fact that the para position is blocked or to the detoxicating effect of the methoxy group. Benzene also gives a rise in the neutral sulphur and it is thus probable that benzene is partly excreted as a mercapturic acid.

**Lactic acid in human muscle.** By A. V. HILL, C. N. H. LONG,  
and H. LUPTON.

In a recent paper (*Quart. Journ. of Medicine*, 16, p. 135, 1923) we have emphasised the magnitude and importance of the rôle of lactic acid in human muscular exercise. One of us therefore (C. N. H. L.) has carried out determinations of the lactic acid appearing in blood as the result of short and violent effort. Clausen's modification of the method of v. Fürth and Charness was employed, a number of preliminary tests being made on ox blood to which a known amount of lactic acid had been added. The average recovery of acid was 85 p.c. A sample of blood was drawn from the arm of the subject immediately after the determination of his resting metabolism. Violent exercise (jumping and skipping) was then carried out for  $1\frac{1}{2}$  minutes, and after 12 minutes' recovery a second sample of blood was withdrawn. Analysis showed that the lactic acid concentration (multiplied by 1/0.85 to allow for loss in manipulation) had risen from 29 mgr. to 104 mgr. per 100 c.c. of blood. The increase is 75 mgr. per 100 c.c. Assuming the blood volume (in the 60 kilo. subject) to be 4.5 litres, this means that 3.4 grms. of lactic acid were present in the blood 12 minutes after  $1\frac{1}{2}$  minutes of exercise. This corresponds to a loss of 18.2 c.c. of  $\text{CO}_2$  per 100 c.c. of blood. Actual determination of the alveolar  $\text{CO}_2$ , five minutes after the blood had been withdrawn, gave a fall of 1.5 p.c. Clearly direct measurement amply confirms our previous high estimate of the lactic acid liberated in short-lived violent exercise: the maximum amount liberated in the muscles must have been many times the 3.4 grms. present in the blood 12 minutes after exercise.

We have been able to show, during oxidative recovery from exercise in man, that lactic acid is removed, and restored to its precursor, with roughly the same "efficiency" as Hartree and Hill on the one hand, and Meyerhof on the other, have recently found in frog's muscle: in other words that the ratio of lactic acid removed to lactic acid oxidised is about 4 or 5 to 1. During the later stages of recovery from exertion in man an excess of oxygen is being taken in, and an excess of  $\text{CO}_2$  given out (*i.e.* above the resting level) in completing the recovery oxidations. There is strong evidence from the isolated muscle that the R.Q. of the recovery oxidations after severe stimulation is unity, indeed that lactic acid itself is being oxidised. Hence the excess of  $\text{CO}_2$  given out should equal the excess of oxygen taken in. It does not; there is a very considerable  $\text{CO}_2$  retention in the body. It is natural to attribute

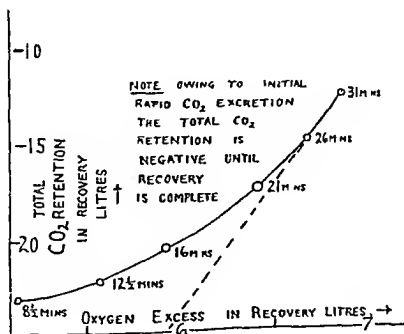
this  $\text{CO}_2$  retention to the combination of  $\text{CO}_2$  with base liberated by the oxidative removal of lactic acid. Hence we may use the  $\text{CO}_2$  retention as a measure of the lactic acid removed, the  $\text{O}_2$  usage as a measure of the lactic acid oxidised: the relation follows,

$$\frac{(\text{lactic acid removed})}{(\text{lactic acid oxidised})} = \frac{3 (\text{CO}_2 \text{ retained})}{(\text{O}_2 \text{ used})}$$

The figure shows an actual experiment. The ratio

$$(\text{CO}_2 \text{ retained})/(\text{O}_2 \text{ used})$$

in the recovery process tends to become constant during the later stages of recovery, i.e. presumably as the hydrogen ion concentration of the blood returns to its normal value. The final value of the ratio in this experiment appears to be about 1.4, so that the ratio (lactic acid removed) (lactic acid oxidised) has the value 4.2. This value is probably too low, since all the base liberated by the removal of lactic acid will not be employed to retain  $\text{CO}_2$ , part of it will certainly be seized by the proteins and other weak acids of the body. It is clear therefore that the main part of the lactic acid produced in exercise is not removed by oxidation, but restored in the recovery mechanism, and it is satisfactory that the complex process of recovery from severe exercise in man is so similar in principle to that discovered in the isolated frog's muscle.



**The ethereal sulphate and mercapturic acid syntheses  
in the dog. By T. S. HELE.**

It has been a matter of controversy for the past forty years whether phenol will combine with sulphate directly in the animal to form ethereal sulphate. Recently Rhode<sup>1</sup> has failed to show in rabbits any union between phenol or bromphenol and sulphate, when the latter is introduced subcutaneously. The failure to demonstrate the direct synthesis was probably due to two causes, (1) the mode of administration of the sulphate and (2) the smallness of the doses of phenol and of bromphenol as compared with daily output of sulphate from the catabolised protein.

If small doses of phenol are replaced by large doses of the non-toxic guaiacol and the sulphate is given by the mouth, direct evidence of their union may be obtained. In a series of experiments on dogs from 60 to 80 p.c. of the administered sulphate was excreted as ethereal sulphate.

In the same way it may be shown that chlorbenzene after oxidation to chlorphenol will unite with sulphate to form ethereal sulphate as well as with cystin to form mercapturic acid.

Is there any numerical relation between the amount of ethereal sulphate and the amount of mercapturic acid excreted? If it is legitimate to regard the excess of neutral sulphur excreted after a dose of chlorbenzene over the average daily output on a standard diet as an estimate of the mercapturic formed, then there is. To dogs, kept on a standard diet, varying doses of chlorbenzene and of brombenzene were administered by mouth. Within experimental limits it was found that the rise in neutral sulphur was always about double the rise in ethereal sulphate. Each molecule of chlorbenzene corresponds to one atom of sulphur whether in the form of sulphate or of mercapturic acid. The mean value obtained showed that 62 p.c. of the chlor- or brom-phenol, excreted in combination with sulphur, was excreted in the neutral sulphur fraction and 38 p.c. in the ethereal sulphate fraction. This constancy holds good for the first day after the dose, when the excretion is maximal.

It was also found very difficult, if not impossible, to upset this quantitative relation by the simultaneous administration of sodium sulphate or of cystin by the mouth. In the latter case 60 p.c. of the cystin was excreted as ethereal sulphate. The cystin was mostly oxidised before it became effective at the seat of synthesis.

<sup>1</sup> *Zeit. f. physiol. chem.* 124. 15. 1922.

Rhode obtained some evidence that the simultaneous administration of cystin subcutaneously with brombenzene given by the mouth gave a smaller yield of ethereal sulphate as compared with brombenzene alone. He was apparently able to increase the effective concentration of cystin at the seat of synthesis. A difference in the method of administration produces a different result.

**The effect of insulin on the respiratory exchange.** By H. W.

DUDLEY, P. P. LAIDLAW, J. W. TREVAN and ELLEN M. BOOCK.

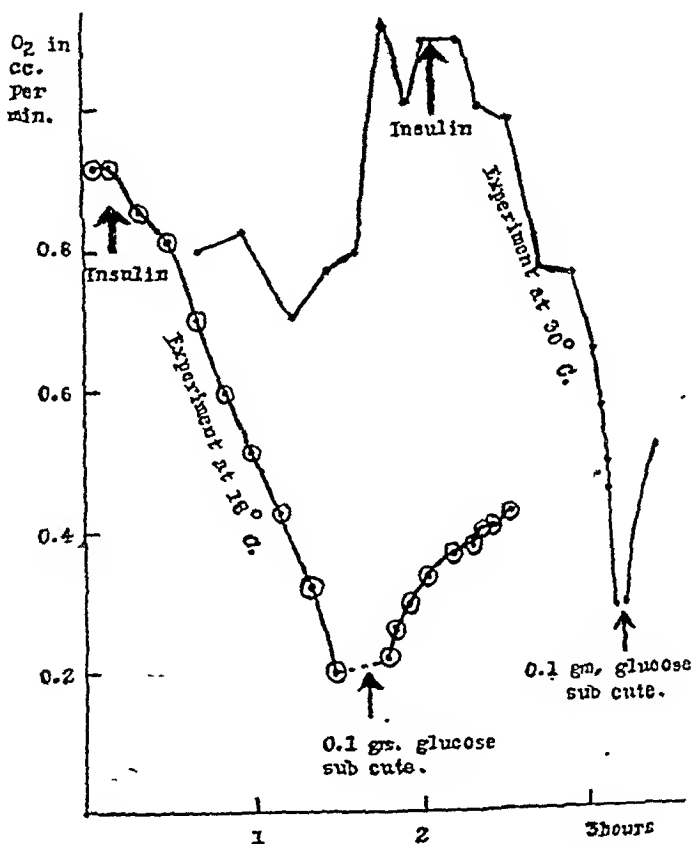
The following is an account of two independent investigations of the effect of insulin on the intake of oxygen and on the output of carbon dioxide by mice.

The output of carbon dioxide was determined by the method of Haldane using the small apparatus recommended by Pembrey and Haldane for air analysis. Mice were used in groups of three. When 0.001 mgm. of an "insulin" preparation per mouse was given the  $\text{CO}_2$  output fell promptly from 10.8 gm. per kilo per hour to 7.35 gm., and at the end of an hour was only 5.29 gm. In another experiment "insulin" and glucose were injected together, and the  $\text{CO}_2$  also fell but more slowly and to a smaller extent (9.73 gm. per kilo per hour to 7.8 gm.). In a further experiment "insulin" was given alone and a fall from 9.16 gm. per kilo per hour to 4.98 gm. promptly developed. A small hypodermic injection of glucose was then given, and in spite of this the  $\text{CO}_2$  output fell to 3.40 gm. per kilo per hour. One c.c. of 5 p.c. glucose was then given and a temporary rise to 4.87 gm. was observed. One hour later the output was down to the very low figure of 3.02 gm. per kilo per hour. Corresponding to this low metabolism the body temperature fell. These low figures were obtained in spite of convulsive movements. The glucose improved the condition of the mice but did not raise the  $\text{CO}_2$  output to figures approaching the normal.

Two experiments were carried out along the same lines on a small rabbit. A fall in  $\text{CO}_2$  output was again recorded (1.57 gm. per kilo per hour to 1.15 gm.). At no stage in any of the experiments was there evidence of increased  $\text{CO}_2$  production.

The intake of oxygen was measured at room-temperature by enclosing the mouse in a small hottle containing soda-lime. To the hottle was attached a water manometer and a 10 c.c. Record syringe containing oxygen. One c.c. of oxygen was introduced into the hottle at intervals

and the time taken for the pressure to return to atmospheric level. Very large doses of "insulin" were injected (0.2 mgm. per mouse). The result was always a diminution in the amount of oxygen absorbed. (See graphs.) At room temperature the metabolism fell gradually from the first. When glucose was injected subcutaneously in large doses (0.1 gm.), a rise in the oxygen consumption was generally produced, but in one experiment, when 0.5 mgm. of insulin had been given, and the metabolism had fallen from 1.3 c.c. per min. to 0.087 c.c. per min. (not plotted), no



rise at all took place after glucose, although the mouse, which was practically dead before the injection of glucose, started to walk about again. The obvious control experiment on the absorbing power of the soda-lime was done by putting a normal mouse into the bottle and obtaining a normal figure.

Experiments were also done at 30.5° C. in an attempt to eliminate any secondary effect of the fall of body temperature (see graph). These were carried out in an apparatus in which the air was constantly circulated

through NaOH and  $H_2SO_4$  after the manner of Benedict. The fall was delayed in onset but was just as pronounced and developed more rapidly when it did start. The convulsive movements did not increase the  $O_2$  absorption in mice. Some experiments were also done on guinea-pigs with similar doses and similar results were obtained in those pigs in which convulsions developed.

These experiments indicate that although the sugar disappears from the blood, the injection of "insulin" does not produce its effect by directly increasing the rate of combustion of glucose. Experiments by one of us (H. W. D.) show that the disappearance is not due to an increased glycogen storage in the liver.

**A new method for the estimation of the ergotoxin content of ergot preparations.** By W. A. BROOM and A. J. CLARK.

The ergotoxin content of ergot preparations can be measured by determining the minimal concentration that will reverse the action of adrenalin upon the rabbit's uterus.

A piece of isolated rabbit's uterus about 1 cm. long is used, its response to 0.0001 p.c. adrenalin is measured, and, after washing out, a known concentration of ergot preparation is introduced. This is left in contact for five minutes, and then the preparation is again washed out, and after five minutes 0.0001 p.c. adrenalin is introduced.

The following is an example of the results obtained:

*Expt. 19. 2. 23. Rabbit's uterus.*

Adrenalin 0.0001 p.c.—contraction 41 mm.

Sample X Liquid extract ergot 0.08 p.c.

Adrenalin 0.0001 p.c.—contraction 41 mm.

Sample X Liquid extract ergot 0.1 p.c.

Adrenalin 0.0001 p.c.—contraction 26 mm.

Sample X Liquid extract ergot 0.12 p.c.

Adrenalin 0.0001 p.c.—inhibition.

On repetition with another strip of uterus 0.12 p.c. of this sample of ergot extract was again found to reverse the action of adrenalin.

The ergotoxin content of different preparations of ergot was found to vary independently of the other active principles, and no certain relation was found between the action of ergot on the guinea pig's uterus and its ergotoxin content.



The following are examples of results obtained on a series of samples of liquid extract of ergot:

*Relative Activities of Ergot Samples.*

Sample	Activity measured on uterus of guinea-pig	Ergotoxin content	
		Estimated on rabbit's uterus	Estimated by action on cock's comb
B	100	20	30
5 A	100	100	100
5 B	30	Nil	Nil
5 C	30	"	"

Ergotoxin was found to produce a reversal at a dilution of 1 in 4,000,000. The sample of ergotoxin was probably not pure.

The three preparations 5 A, 5 B, and 5 C were made from the same sample of ergot:

5 A	according to the	United States Pharmacopœia.
5 B	" "	British Pharmacopœia.
5 C	" "	French Codex.

**The influence of carbonates on the heart rate.**

By HENRIETTA BAINBRIDGE.

Mansfeld and Szent-Györgyi(1) recently studied the effects of perfusing the frog's heart with dilute solutions of alkalis, and concluded that carbon dioxide was the natural stimulus which provoked the rhythmic automaticity of the heart and that the free carbon dioxide in Ringer's solution acted as an accelerator to the heart. They showed that perfusion with .002N solutions of  $\text{Ba}(\text{OH})_2$ ,  $\text{NaOH}$ ,  $\text{NH}_4\text{OH}$  and  $\text{Na}_2\text{CO}_3$  (in Ringer of the following composition:  $\text{NaCl}$  .6%,  $\text{KCl}$  .01%,  $\text{CaCl}_2$  cryst. .02%) caused disturbance of the normal heart beat and provoked idiopathic auricular beats, nodal rhythm and, finally, arrest of the heart; also that  $\text{NaHCO}_3$  and  $\text{NaHPO}_4$  in this Ringer in .002N concentration did not produce this effect, and concluded that this proved that the action of the heart did not depend upon  $\text{OH}$  ion concentration. This conclusion is unjustified, for a .002N solution of  $\text{NaOH}$  has a  $\text{pH}$  of about 11.5, a .001 solution of  $\text{Na}_2\text{CO}_3$  a  $\text{pH}$  of about 11, while .002N solutions of  $\text{NaHCO}_3$  or  $\text{Na}_2\text{HPO}_4$  have a  $\text{pH}$  of about 9. The action of  $\text{NH}_4\text{OH}$  and of  $\text{Ba}(\text{OH})_2$  are not comparable as both  $\text{NH}_4$  and  $\text{Ba}$  ions are strong heart poisons.

Dale and Thacker(2) showed that the limit of alkalinity at which the various portions of the heart of the frog could function was as follows: sinus,  $\text{pH}$  9.5, auricle, 10.5, ventricle, 11.0. The experiments of Mansfeld and Szent-Györgyi merely confirm these results; solutions

of  $\text{NaHCO}_3$  and  $\text{Na}_2\text{HPO}_4$  whose  $p_H$  is below 9.5 do not arrest the sinus beat, whilst solutions of  $\text{NaOH}$  etc., with a  $p_H$  of about 11 arrest the sinus and first stimulate and finally arrest the ventricular beat.

I have made experiments comparing the action of ordinary carbonate containing Ringer with the action of the solution described by Mines (3), in which boric acid and sodium acetate are used as buffers and the fluid is carbonate free. Hearts of Ran. temp were perfused by means of a Symes cannula. The solutions were made with water freshly distilled from glass, and, in the case of Mines' solution the water was boiled to remove  $\text{CO}_2$ . The  $p_H$  of both fluids was carefully adjusted to 7.7. The following table shows typical results.

	Ringer's Fluid			Mines' Fluid		
	Beginning of experiment	3 hrs later	18 hours later	Beginning of experiment	3 hrs later	18 hours later
Rate per min	36	29	17	31	26	22
Height of contractions in cm	0.83	0.76	—	0.95	0.70	0.1
Volume output in c.c. per min	0.9	1.0	—	1.9	1.6	0.1

These results show that the frog's heart beats at the same rate when perfused by a fluid devoid of carbonates as it does when perfused by one containing them.

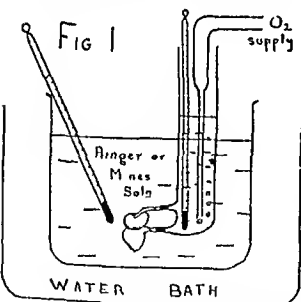


Fig 1 Apparatus used, which was placed in a water bath

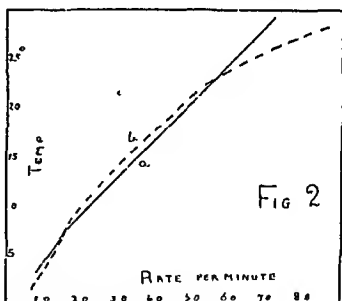


Fig 2 Heart rate  $\alpha$ , in normal Ringer,  $\delta$ , in  $\text{CO}_2$ -free Mines' solution

Further experiments were made to study the effect of temperature on the rate in the two conditions. A double cannula as shown in Fig 1, was used and the heart plunged into a bath of Ringer or Mines' solution at varying temperatures, the cannula being filled with the same solution at the same temperature. Rates were measured by counting the auricular

**Canalicular appearances within nerve-cells of the spinal cord, spinal and sympathetic ganglia in vitamin B deficiency<sup>1</sup>.** By C. DA FANO.

The appearances here described were noticed in nerve cells of the spinal cord, spinal and sympathetic ganglia of the animals to which reference was previously made, but chiefly of pigeons kept on polished rice and cats fed on autoclaved meat. While under such conditions the Golgi apparatus stained about as usual and the Nissl substance seemed only reduced in quantity, the cytoplasm of most nerve cells was pervaded by an apparent system of canaliculi similar to, if not identical with, Holmgren's trophospongium. In some cells only a few whitish streaks were seen in their most peripheral portions; in others the whole cell-body was permeated by the apparent canaliculi. These looked, in general, like stainless spaces of changing shape, appearing sometimes as minute and relatively straight fissures, sometimes as variously bent canals. In a certain number of cells the spaces were so wide as to impart to the cytoplasm an almost vacuolated aspect, some of the vacuoles surrounding part of the nucleus. In many cases the canals or spaces opened at the edges of the cell-bodies, thus freely communicating with the surrounding tissue. The examination of a great number of specimens from differently fixed and differently embedded pieces showed that the phenomenon was independent of the technique, but perhaps connected with a certain degree of congestion and oedema frequently present in the nervous tissue of animals affected by vitamin B deficiency.

Facts of the same kind have been previously observed<sup>2</sup> but, as far as I know, not in deficiency diseases and not so plainly. In consideration of this, particular attention was paid to specimens treated by the cobalt nitrate method in order to find out in which relation the apparent canaliculi and the Golgi apparatus stood to each other. Many cells were thus observed in which apparatus and canals or spaces were clearly visible at the same time. This observation shows in a definite manner that the Golgi apparatus and the so-called Holmgren trophospongium are entirely distinct from each other. It is worth adding that in some cells of the spinal ganglia the apparent canaliculi seemed to contain some material on the nature of which no definite opinion can be at present expressed.

<sup>1</sup> The expenses of this investigation have been defrayed by a Grant from the Medical Research Council.

<sup>2</sup> See Da Fano. *Journ. Nerv. Ment. Dis.* 53. p. 353. 1921, and the references therein contained.

**A method of demonstrating changes in the calibre of the bronchioles.** By R. J. S. McDOWALL.

The method consists essentially of using the chest cavity as a plethysmograph and recording changes of lung volume during constant artificial respiration.

The muscles are retracted from the side of the sternum about the fifth interspace. An opening is made into the chest, the little finger inserted to tear the pleura, a flanged cannula inserted and attached to a volume recorder. The muscles are allowed to slip back and the wound made airtight by a purse-string suture. The descent of the diaphragm is reduced by bandaging the abdomen but it is not usually necessary to take any steps to immobilise the chest wall.

In this way changes in bronchiole calibre may be shown which are difficult to demonstrate by the tracheal-pressure method, and the use of delicate oneometers is avoided.

**Non-protein nitrogen and urea content of the blood during pregnancy.** By E. CHRISTINE PILLMAN WILLIAMS.

Any worker undertaking an investigation of abnormal cases of pregnancy is at once faced with the lack of information as to the change which takes place in the blood during normal pregnancy. Chemical analysis of the blood of normal non-pregnant women gives as average values:

Non-protein nitrogen	30 mgm. per 100 c.c. of blood,
Urea ... ..	25-30 mgm. per 100 c.c. of blood.

Folin from the analysis of about 100 pregnancy cases finds that the N.P.N. is rarely over 30 mgm. per 100 c.c. of blood, while the urea varied from 10.7-19.2 mgm. Caldwell and Lyle found much higher values for their series of 150 cases, *e.g.* N.P.N. 29.59 mgm. p.c., urea 24.6 mgm. p.c., and found slightly higher values for the 9th month. De Wesselow, examining about 50 normal women, finds values corresponding to those of Folin.

During the last two years the writer has been making careful examination of the blood content of normal pregnant women with a view of coming to some definite conclusion as to whether there is any variation of the blood urea with the different months of pregnancy.

In the following series two or more examinations of the blood have been made in each case. The average figures during the whole term of pregnancy (from 100 analyses) have been found to be

N.P.N. 25.2 mgm. p.c. Urea 19.5 mgm. p.c.

being particularly affected. In the end they seemed almost incapable of standing and if compelled to walk they moved their hind limbs inordinately, and frequently fell on one side or the other. But they did not show true paralytic symptoms as seen, for instance, after the section of a nerve. Only one of these animals seemed unable to retract its claws completely. In cats, as in rats, no proof of any sensory disturbance was obtained.

Pieces of the spinal cord, a certain number of spinal ganglia and in some cases the largest sympathetic ganglia were investigated by the methods in use for the demonstration of Golgi's apparatus and Nissl's substance, particular attention being paid to specimens from the spinal cord, spinal and sympathetic ganglia of cats in which a degeneration of peripheral and central nerve fibres had been found. As regards the Golgi apparatus no determined lesion could be seen in any of the many preparations examined. It stained as well and possessed the same characteristic structure as in similar cells of normal animals. A somewhat unusually fragmented appearance was seen in such few instances that no special importance could be attached to it. As regards the Nissl substance it seemed reduced in quantity in many cells of the spinal cord and ganglia, particularly of cats; but true chromatolytic changes with peripheral shifting of the nucleus were noticed only occasionally in a very small number of cells.

### **Action of guanidine salts on dilute glucose solutions.**

By G. A. CLARK. (*Preliminary Communication.*)

It has been shown that the effect of pure guanidine on glucose in aqueous solution is essentially similar to that of an alkali<sup>1</sup>. In attempting to determine the effect of guanidine salts on the estimation of glucose in dilute solution, evidence of other interaction was found. The addition of .1 gm. guanidine hydrochloride per 100 c.c. of sterile glucose solution caused a definite diminution of copper reducing power as determined by Bang's old micro-method for blood-sugar. The minimum reducing power was attained in 30 minutes in average bright daylight, but in solutions kept in the dark no change was observed even after four days. Exposure to daylight for 10 minutes or more, however, resulted in diminished reducing power even when the solution was subsequently kept in darkness. This altered reducing power could not be detected by Maclean's method of blood-sugar estimation.

<sup>1</sup> *Journ. Chem. Soc.* 1907. *Trans.* p. 1010.

Exp 7 (Table I) suggests that the full reducing power is restored by prolonged boiling

TABLE I

	Daylight		Dark	
	Glucose only	Glucose + guanidine salt	Glucose only	Glucose + guanidine salt
1	060	047	—	060
2	067	052	—	070
3	080	062	080	080
4	105	087	105	105
5	157	137	157	157
6	167	147	—	170
7	062	22 " 23 046	060	062
	060	23 " 23 045	060	062
		Boiled in dark 10 mins		Exposed to daylight
		Cooled, made up to original volume Kept in dark overnight	20 mins	052
			30 "	047
	060	24 " 23 062	45 "	047

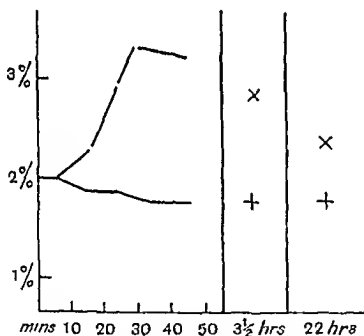


Fig 1

x — — Polarimeter value  
+ — — Bang value  
Both expressed as p c glucose for comparison

Comparison of optical activity with copper reducing value showed an initial increase in rotatory power simultaneous with the fall in copper value. Thereafter the rotation diminished, and fell to that of the original glucose solution in from 1 to 3 days (see Fig 1). Solutions previously kept in the dark also showed these changes on exposure to daylight.

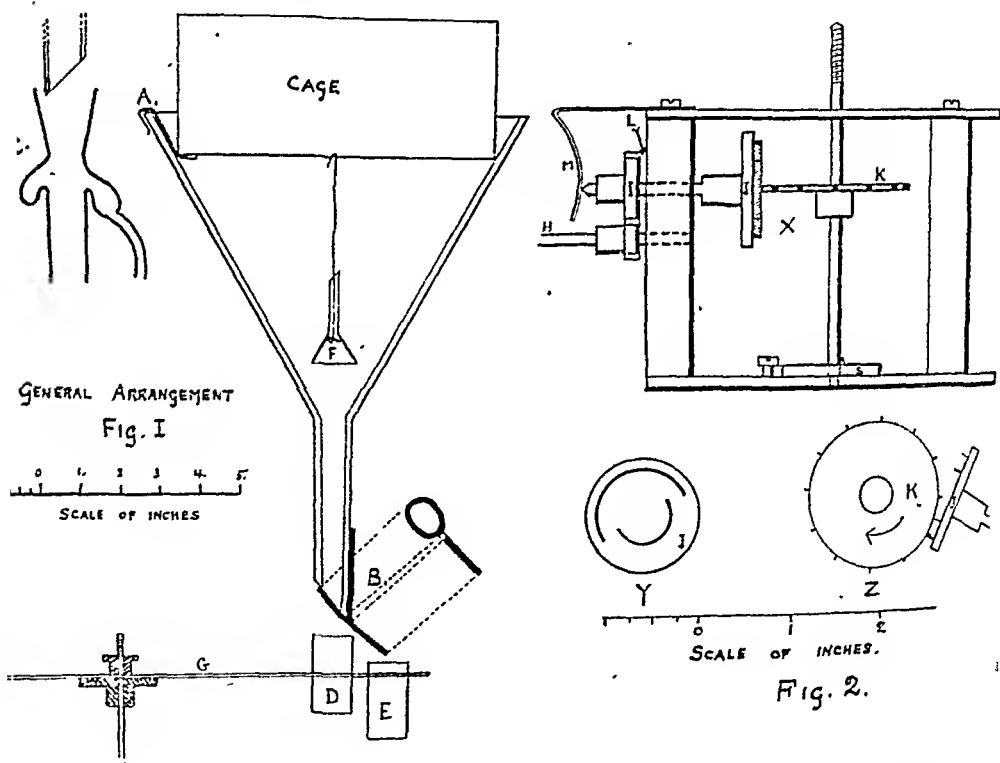
Using guanidine carbonate or sulphate instead of the hydrochloride similar results were obtained. These results cannot be due to the forma-

tion of the guanidine-glucose compound whose properties were investigated by Morrell and Bellairs<sup>1</sup>, because this compound showed a specific rotatory power considerably less than that of pure glucose.

I am indebted to Professor D. Burns for the supply of pure glucose and guanidine salts, and to Professor H. V. A. Briscoe for the loan of the polarimeter.

### **Separation of excreta from rats. By LOUIS GROSS<sup>2</sup> and S. J. B. CONNELL.**

The simple apparatus shown below automatically times and separates the excreta from rats.



The general arrangement is shown in Fig. 1. A cylindrical rat cage rests on three copper hooks (A) which are hung from a large glass funnel. When the feces and urine fall through the large wire mesh at the bottom of the cage, the feces drop straight through the neck of the funnel into

<sup>1</sup> *Journ. Chem. Soc. 1907. Trans. p. 1010.*

<sup>2</sup> Beit Memorial Research Fellow. Aided by grants from the British Medical Association and the Medical Research Council.

small receiving cups (*D*). The urine flows down the sides of the funnel and strikes the small oval "tennis racquet-shaped" glass rod (*B*) whence capillary attraction allows it to flow to the point. Here it falls into the receiving cups (*E*). A small inverted funnel (*F*) is suspended from the centre of the cage in order to divert urine from falling directly through the large funnel.

Instead of the "racquet-shaped" conductor (*B*), one can use the even more efficient separator shown in section in Fig. 1 c. This requires no adjustment and has the added advantage that the urine is easily and safely conducted to any receptacle. In this way one can make use of the row of cups (*E*) to collect feces from another rat. This separator consists in a bulb of the form shown. A tube leading from the lowest point of the moat serves to remove the urine, while the feces drop straight through.

The tubes (*D*) and (*E*) are arranged in a circle on a horizontal disc (*G*) having two or more rows of 24 holes each, cut at equal intervals on a circumference where they are conveniently held in position by slipping rubber bands over them. The motive power is supplied by a clock spring Fig. 2 (*S*) which, when wound, rotates the disc and brings the cups to the correct position.

The timing is carried out by attaching the rod (*H*) through a universal coupling to the minute hand of a clock. This makes one revolution in one hour. By gearing it to a wheel (*I*) of appropriate size, one can obtain one revolution of (*I*) in any desired interval.

The actual control of the movement of the disc (*G*) is shown in (*X*), (*Y*) and (*Z*) which are elevation, face view and plan respectively. A wheel (*J*) has two raised rings formed on its face, cut away to form two slightly overlapping segments as shown in (*Y*). The radial distance between the two segments is equal to half the distance between the teeth of the engaging twelve tooth wheel (*K*). As shown in (*Z*), one tooth of (*K*) is resting against the outer segment of (*J*). As (*J*) rotates, this tooth slips off the end of the segment and the next tooth is caught by the inner segment in the position shown dotted in (*Z*). Another half revolution of (*J*) allows this tooth to slip onto the other segment and the process is repeated again. It will be seen that for every revolution of (*J*) the wheel (*K*) makes two movements, each  $1/24$ th of a revolution.

The apparatus as shown is arranged to change every hour. The wheel (*I*) is twice the size of that on (*H*) and therefore (*J*) rotates once every two hours, so that (*K*) moves once every hour bringing a fresh tube into position below the funnel.



To wind the spring (*S*), a wedge shaped lever (not shown) is introduced at (*L*) which withdraws (*J*) from (*K*), leaving the latter free. The disc is then rotated one complete revolution in the direction which tightens the spring (*S*). The lever is then released from (*L*), (*J*) is pushed into engagement with (*K*) by the brass strip (*M*), and the disc is locked in position ready for use.

# PROCEEDINGS

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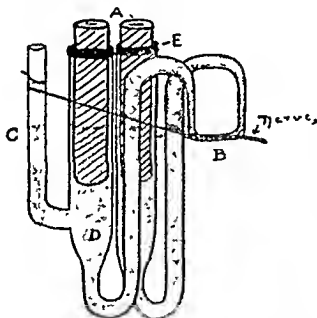
# PHYSIOLOGICAL SOCIETY,

*May 19, 1923.*

### A new type of electrode. By HENRY DRYERRE.

In prolonged experiments in the investigation of the response of an exposed nerve to intermittent stimulations, difficulties occur in the maintenance of a uniform degree of excitability in the nerve. Variations in the moisture of the latter, in the site of application of the electrodes, and damage by frequent manipulation are contributing factors towards irregular results.

Keith Lucas<sup>(1)</sup> utilised "fluid" electrodes in his experiments with dissected nerve-muscle preparations, but his electrodes could not be



applied to divided nerves *in situ*. In seeking to overcome these difficulties I have evolved a new type of electrode. This electrode allows the stimulus to be applied to the same part of the nerve throughout an entire experiment; the nerve suffers no mechanical injury from the application of the electrode; the moisture is maintained constant for many hours if necessary; and the electrode is non-polarisable. In addition, the portion of the electrode in close proximity to the tissues is relatively small, and the principle may be utilised for application to a nerve in any situation.

The sketch shows the electrode used by me for stimulation of the cervical vagus nerve of the cat and rabbit. It is made of glass through-

out, with the exception of the two plugs *A*, which are made from pieces of clay pipe-stems having their bore widened and the lower end plugged with plaster of Paris. These contain zinc sulphate solution and into them dip amalgamated zinc terminals. The plugs are maintained at the proper level in the tubes *D*, by means of the adjustable rubber collars *E*.

The rest of the electrode is filled with Ringer's solution. *B* is a horizontal limb which lies in the sulcus made in dissecting out the nerve. This limb has a hole at either end, and through these holes the divided nerve is drawn by means of an attached piece of thread. This thread is fixed by plasticine to the upright tube *C*, which latter serves the double purpose of forming a convenient part of the electrode to be fixed in a clamp, and as an inlet by which any loss of Ringer's solution may be replaced.

The shape of the limbs, and the level of the Ringer's solution in the tubes *D* have been so adjusted that there is no undue tendency for fluid to escape from the electrode.

#### REFERENCE.

- (1) Keith Lucas. Journ. Physiol. 1906, 34. 374; 1908, 37. 114.

#### **Antidromic action.** (*Preliminary Communication.*)

By J. N. LANGLEY.

In recent experiments on a possible action of posterior nerve roots on sweat secretion, I noticed the flushing of the foot first described by Morat about 30 years ago. The obviousness of the flushing suggested that by stimulating the posterior roots after section of one or more of the nerves of the foot near the periphery, it could be determined whether the whole vascular tract from the femoral artery downwards or only the extreme ends are affected by antidromic impulses. Discussion of the results requires a knowledge of the complex arrangement of the nerves and blood vessels in the foot, which cannot be given here. The conclusions I draw from a number of varied experiments is that the arteries, except possibly the small branches given off by the digital arteries, are not appreciably affected by antidromic impulses and that the flushing is chiefly due to an action on the capillaries. The balance of indirect evidence is, I think, in favour of the flushing being due to metabolites, and not to nervous action on the vessels, but direct evidence of this has not been obtained. Flushing in one part of the foot is commonly accompanied by pallor in the rest of the foot; suggesting a great effect of local arterial pressure on capillary diameter.

**A modified form of Martin's Bicycle Ergometer, by the Designs Department of the W.D. Experimental Station, Porton.**

The instrument consists of an ordinary bicycle held up securely on a wooden stand, the front wheel removed, its forks held securely to the stand and the back wheel replaced by a heavy flywheel with a broad rim. A long leaf of spring steel is secured at its "steering head" end to the floor of the stand; the other end is free to float but has attached to it a broad band of canvas which is laid around the rim of the flywheel.

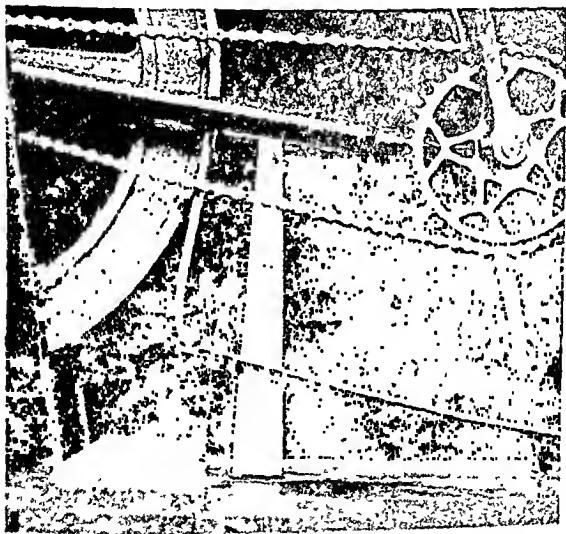


Plate I.

Secured to the tail of the band, and hanging from it, is a weight whose action is to deflect the end of the leaf spring upwards. Standing up from the floor of the stand is a fixed strip of brass plate forming a scale, and by varying the tail weights the spring can be calibrated and the scale marks added to the brass strip. See Plate I.

On the left side of the apparatus there is fixed to a convenient part of the stand a revolution counter which is worked by a projecting pin from the flywheel, a safety device being incorporated on the actuating

lever of the counter to prevent any damage if the wheel is revolved backwards by accident.

When the machine is pedalled (see Plate II) the friction between the rim of the flywheel and the canvas band (which is constant, and independent of rate of pedalling) acts against the weight and reduces the deflection of the spring by an amount which can be read off on the scale which is marked so as to give the difference between the tensions on the tight and slack ends of the band for a given tail weight. This reading is theoretically constant, but actually it is advisable to check it occasionally if the machine is used a great deal.



Plate II.

This reading, then, together with the reading of the counter, give all the requisites to calculate the work done, thus the work done in ft. lbs.

$$= \text{Reading} \times \text{Circumference of wheel} \times \text{No. of revolutions.}$$

Each weight used on the tail should have its corresponding spring and scale if the readings are to be sensitive. A series of weights, scales and springs can be made in sets, each set to suit its particular rate of work.

The machine has the following advantages over Martin's type. It is simpler, there are fewer parts to get out of order or need adjustment, the readings of tension are taken directly off the end of the canvas band and there is no sliding friction of the spring balance pointers to introduce errors.

**The removal of lactic acid during recovery from muscular exercise in man. By C. N. H. LONG and H. LUPTON.**

That the lactic acid produced by stimulation of an isolated muscle and removed in recovery is not all oxidised has been decisively demonstrated by Meyerhof, and by Hartree and Hill; according to the former  $\frac{2}{3}$  to  $\frac{3}{4}$  of the lactic acid is restored to glycogen in a process by which the remainder is oxidised, according to the latter about  $\frac{1}{2}$  to  $\frac{5}{8}$ . It was of interest therefore to determine in man, the ratio of lactic acid removed to lactic acid oxidised. A method described in a previous communication, employing  $\text{CO}_2$  retention after exercise as a measure of total lactic acid removed, and excess  $\text{O}_2$ -usage as a measure of lactic acid oxidised, gave a ratio of about 4 : 1. In the present method the lactic acid removed is directly measured, that oxidised determined as before from the excess  $\text{O}_2$ -usage.

*Procedure.* After a determination of the oxygen consumption at rest, fasting, the subject ran at a speed too great to be kept up indefinitely: exercise was prolonged sufficiently (8 minutes and upwards) to cause large quantities of lactic acid to enter the blood. This was followed by violent skipping and jumping, after which the subject adopted the original resting position. Earlier experiments on recovery from exercise had shown that, for a period of at most 10 minutes carbon dioxide is excreted in excess of what could have been produced by contemporary oxidation of carbohydrate. Lactic acid was thus obtaining base from bicarbonate in blood and tissues.

For this period at least, therefore, the lactic acid is not evenly distributed throughout the soft tissues of the body. After about 10 minutes of recovery a prolonged period of  $\text{CO}_2$  retention sets in. This shows that bicarbonate is being restored. Consequently, we felt justified in assuming that, after about the first 10 minutes of recovery the concentration of lactic acid in the blood would, at any instant, be the same as in the rest of the tissues, and could therefore be taken to represent the concentration of lactic acid throughout the body. To make sure that this state of equilibrium was attained the first sample of the blood was not taken, at the earliest, until after the first 15 minutes of recovery. Experiments are now in progress to determine the time which must elapse, after the end of exercise, for the concentration of lactic acid in the blood to be a maximum. To determine the lactic acid removed, two samples of venous blood, each about 5 c.c., were taken during the recovery period following the severe exercise, an interval of half an hour being allowed to elapse between the two samples. These were analysed for lactic acid,

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# PHYSIOLOGICAL SOCIETY,

*July 7, 1923.*

**The formation of "breakers" in the transmission of the pulse-wave. By J. CRIGHTON BRAMWELL<sup>1</sup> and A. V. HILL.**

It has been shown(1) that, in an isolated artery, the velocity of the front of the pulse-wave varies with the pressure; and since, in the living body, the blood-pressure rises rapidly during the initial stages of the pulse cycle, different elements of the pulse-wave will be transmitted to the periphery with different velocities. The front of the pulse-wave is launched on the blood stream at the diastolic pressure, and travels at

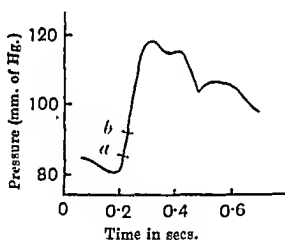


Fig. 1.

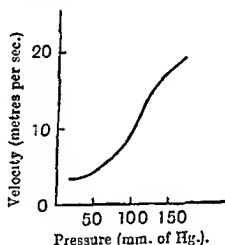


Fig. 2.

Fig. 1. Pressure-time relation in initial stages of normal carotid sphygmogram.

Fig. 2. Velocity-pressure relation, as determined in isolated carotid artery.

the slowest rate; the part of the wave which travels most rapidly is that which is launched at the systolic pressure: intermediate parts travel at intermediate rates. Hence, the more rapidly moving elements will tend to catch up those which are moving more slowly; in other words the wave will tend to become more vertical, and, under suitable conditions, parts of it may actually tend to topple over and form "breakers." Even if the wave as a whole does not "break," any individual portion of it which does so will set up irregular vibrations in the arteries, which, if they be of sufficient amplitude, will be transmitted to the periphery. Vibrations, which it is suggested may be produced in

<sup>1</sup> Working for the Medical Research Council.



this way, have been demonstrated both by optical methods(2)(3), and by the hot wire sphygmograph(4). Similar vibrations occur on the upstroke of sphygmograms obtained from normal subjects immediately after violent exercise.

In Fig. 1, which is an optical sphygmogram taken from an artery at a point  $X$ ,  $a$  and  $b$  are the beginning and end of an element of the pulse-wave, separated from one another by a time interval  $t$  secs. Since this is a pressure-time curve in which the commencement and top of the upstroke correspond to the diastolic and systolic pressures respectively, the pressures corresponding to the points  $a$  and  $b$  may be read off; and further, the velocities corresponding to these pressures can be ascertained from the curve (Fig. 2) relating velocity to pressure in 'normal' isolated arteries(1). If these velocities be  $v_1$  and  $v_2$  cm. per sec., then the length of the element will be  $t \cdot \frac{v_1 + v_2}{2}$  cm. Hence the time taken by  $b$  to overtake  $a$  will be  $t \cdot \frac{v_1 + v_2}{2} / (v_2 - v_1)$  secs., and the distance which the whole element will travel in that time is  $t \cdot \left( \frac{v_1 + v_2}{2} \right)^2 / (v_2 - v_1)$  cm.<sup>1</sup> If this distance be less than the distance from  $X$  to the periphery, then the element will have time to "break," before reaching the capillary area.

Those pathological conditions in which the diastolic pressure is low, and the pulse pressure is increased, especially if the rise in pressure takes place suddenly, should theoretically offer the most favourable opportunity for the formation of "breakers."

It is possible to study the behaviour of *any* small element on the wave front, and so to ascertain the parts of the wave which would be the first to "break." A simple formula can be devised for this purpose.

It is suggested that the conditions outlined above closely resemble those which are known clinically to be associated with the "water-hammer" type of pulse, and that "breaking" of the pulse-wave may be an important factor in the production of that phenomenon.

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<sup>1</sup> In actual practice, this calculation may require to be modified, in order to take account of the probable fact that, in the carotid, the relation of pressure to velocity is not the same as in the smaller, and more muscular, arteries.

**The minute volume of the heart in respiratory obstruction.**

By A. S. G. HUGGETT

An attempt has been made to find the effect on the circulation rate of causing animals to breathe against a pressure. Urethanised cats were used. It was found that by giving it four or five hours before the beginning of the experiment a uniform anaesthesia was obtained, and apart from a little ether for induction no more volatile anaesthetic was required.

The minute volume was given by the ratio  $\frac{O_2}{A-V}$ , where

$O_2$  = Oxygen consumption per minute, and

$A-V$  is the difference in oxygen content per c.c. of arterial and venous bloods, i.e. the oxygen utilisation

A cannula was inserted in the trachea and the inspired and expired airs separated by valves. The expiratory valve was a rubber membrane over a slit in glass tubing. The inspiratory valve was a wash bottle with wide glass tubing. By pushing the tubing into the water a pressure could be set up to oppose inspiration and be varied as desired.

The oxygen consumption was measured by collecting the expired air in a rubber bag of about 2000 c.c. capacity, analysing it in a Haldane gas analysis apparatus and measuring the volume with a gas meter.

The difference in oxygen content was measured directly with a Barcroft differential blood-gas apparatus. The arterial blood was collected by a cannula in the femoral artery, the mixed venous blood was obtained direct from the right heart by the method of Barcroft, Boycott, Dunn and Peters<sup>1</sup>, that is cardiac puncture with a hypodermic syringe.

The inspiratory obstruction was a constant pressure of 5 cms. of water. Two readings were taken while at rest before the obstruction, two or three during the obstruction and two after the obstruction.

The average values for nine experiments were:

**A. Oxygen consumption per minute per cat:**

At rest before obstruction	...	21.65 c.c.
During obstruction (5 cms.)	..	22.80 "
At rest after obstruction...	.	21.50 "

**B. Oxygen utilisation per c.c. of blood.**

Before	..	..	0.0706 c.c.
During	...	..	0.0548 "
After..	...	...	0.0730 "

<sup>1</sup> Barcroft, Boycott, Dunn and Peters. *Quart. J. Med.* 13. p. 35. 1919

## C. Minute volume :

Before	...	324 e.e. or	...	130 c.c. per kilog.
During	...	446 e.e. "	...	178 c.c. " "
After	...	300 c.e. "	...	120 c.c. " "

The figure of 130 c.c. per kilog. body-weight agrees with that of Barcroft and his collaborators for the unanæsthetised goat (133 c.c.). It will be noticed that the resting value for the oxygen utilisation is considerably higher than the value stated to occur by Henderson<sup>1</sup> and obtained by indirect methods.

**The initiation of the state of hibernation in mammals.**

BY F. BUCHANAN.

(i) An ordinary mammal becomes poikilothermic if its temperature is once reduced below a certain level (25° C. for the monkey, 24° C. for the cat), and it will recover, though not without some assistance, as long as its temperature does not fall below a certain minimum (16° C. for the cat). This has been shown by Sutherland Simpson and Herring<sup>(1)</sup> who reduced the temperature of the animal in the first instance by deeply anæsthetising it in a very cold environment (4° C.). They used ether as the anæsthetic. (ii) Another way of producing anæsthesia is by deficiency of oxygen-supply to the brain, and this may be produced, in man, either by deep forced breathing or by very rapid shallow breathing (Haldane). My suggestion is that those mammals which hibernate utilise this method of rendering themselves unconscious when their usual form of food is unobtainable, and, when subjected to a low external temperature, become poikilothermic, Natural Selection having favoured those which could best acclimatise themselves to deficiency of oxygen.

Additional facts which support this view are: (iii) The temperature of a homoiothermic animal falls when its oxygen-supply is reduced gradually, e.g. that of a pigeon may fall from 41° to 31° C. (Cl. Bernard<sup>(2)</sup>). (iv) Some, and perhaps all, hibernating mammals, each time they give up the attempt to maintain their temperature, show two phenomena which are associated with want of oxygen in other mammals. One of these, known in man as a symptom of anoxæmia, is the periodic breathing which Pembrey<sup>(3)</sup> has recorded in dormice, bats, hedgehogs and marmots. The other is the heart-block shown in my electrocardiographic records taken with hibernating dormice and bats<sup>(4)</sup>; and Mathison<sup>(5)</sup> has demonstrated that this condition is produced in anæsthetised, decerebrate, and in spinal cats, by insufficient oxygen-supply to the heart.

<sup>1</sup> Y. Henderson. *Physiological Reviews*, 3, p. 165. 1923

At what stage of going into torpor these phenomena first appear has not yet been determined, and would be difficult to determine. But while coming out of torpor, and after the breathing has started and become regular, I find that in certain individual dormice it becomes periodic (and may do so more than once on each occasion of waking) and that whenever this happens there is a relapse into, or in the direction of, the torpid state. Thus the pulse-rate (i.e. the rate of the ventricular beat), which may to some extent be regarded as a measure of the waking, always ceases to rise, or may even fall, each time the periodic breathing begins; whereas the pulse-rate rises without interruption in other dormice which show no periodic breathing while waking. The great difference in the readiness with which individuals of the same species go into and come out of hibernation has been noted by most authors. Such difference manifests itself even in animals kept simultaneously under identical conditions, and in my experience with dormice, one that does so readily always does so, winter after winter. It is only such a one which shows periodic breathing while waking. Individuals which hibernate with difficulty always wake quickly, their pulse-rate rushing up from about 30 to about 600 a minute in the course of an hour.

One way of testing my view would be by carrying further the Haldane breathing experiments, and seeing whether a state of true hibernation can be thus induced in a species in which it is not a normal occurrence. Possibly this experiment has already been made by the Hindoos, since it is known that a Yogi prepares himself for temporary entombment by special breathing exercises, though it is, unfortunately, not yet known whether he loses control of his body-temperature while in the trance. Such an occurrence might escape ordinary observation in a country where the external temperature is not far removed from, and may be higher than, the usual body-temperature of man.

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#### The heat of combustion of glycogen. By W. K. SLATER.

The heat of combustion of the hydrated form of glycogen  $[(C_6H_{12}O_6)_n]$ , prepared as described in the *Proceedings* of this Society for 17th February, 1923, has been determined. A Mahler-Cook bomb calorimeter was used for the estimation. The average for 5 determinations was 3878 cal. per

gram., the values all lying between 3870 cal. per gram. and 3884 cal. per gram. This value is higher relatively than Stohmann's value (4190 cal. per gram.) for the supposedly anhydrous material, due in the author's opinion to the fact that Stohmann was not dealing with completely anhydrous glycogen.

The new value for the heat of combustion of glycogen is of considerable physiological interest, as by subtracting the figure obtained for the heat of wetting and solution (9 cal. per gram.), we can get the energy value for glycogen in solution (3869 cal. per gram.), and thus recalculate more accurately from chemical and thermo-chemical data the heat exchanges in muscular work. The results so obtained are in excellent agreement with the direct measurements of Hartree and Hill.

**Note on the alleged growth promoting effect of air irradiated with the quartz mercury vapour lamp.** By T. A. WEBSTER and LEONARD HILL.

At a recent meeting of the Bio-Chemical Society E. M. Hume and H. Henderson Smith<sup>1,2</sup> reported some experiments now published on the effect of irradiated air on the growth of rats. Rats of 50 gm. weight, kept singly in glass jars and fed on a diet deficient in fat soluble vitamins, were on every second day taken out of their jars and the jars then exposed for ten minutes to the radiations from a quartz mercury vapour lamp, each open jar being placed under the lamp so that the radiations passed directly into the jar without having to travel through the glass. At the end of ten minutes each jar was covered with a glass lid and taken from the lamp—the rat was introduced and the lid replaced for ten minutes, after which the lid was removed and the rat lived in the jar till the next treatment. Control experiments were done in which the jars after irradiation were blown out with a bellows before introducing the rat. As the result of this treatment with irradiated air, according to Hume and Smith, the rats showed a more rapid and prolonged growth compared with the control rats. Their work was suggested by the work of Kestner on the regeneration of erythrocytes in dogs. Kestner used the open carbon arc and found that if dogs which had been made anæmic breathed the air in which the lamp was burning, there was produced a more rapid regeneration of erythrocytes than in control dogs. Kestner thought this might be due to effects of oxides

<sup>1</sup> *Biochem. Journ.* 17. p. 364. 1923.

<sup>2</sup> *M. R. C. Special Report Series.* No. 77. p. 180.

of nitrogen produced by the arc. It is claimed that the fall of blood pressure seen in patients exposed to the carbon arc is prevented by their breathing of pure air. Hume and Smith admit that the only known effect on air of the mercury vapour lamp radiations is the production of ozone and slight ionisation. They show that ozone has if anything a retarding influence on growth. Without committing themselves to any definite statement they suggest by implication that the ionised air is the active growth producing agent.

The statistical evidence on which they base their conclusion is not so strong as it might be. While their graphs are striking, there is one in which controls and experimental rats do not show much difference in growth. This graph illustrates the growth of rats which were first treated for one hour daily to air that had been drawn from near the mercury vapour lamp and then, after fourteen days or so of this, were treated by the glass jar method. The controls in every instance show good growth while the experimental rats showed poor growth. Hume and Smith merely remark that these control rats "showed remarkably strong growth."

We have repeated Hume and Smith's work following their technique as nearly as possible, the only difference being the type of lamp used. Their lamp made by the Hewitt Electric Company consumed 3.5 amps.—our's was a self-lighting lamp made by Kelvin, Bottomley and Baird and consumed 2 amps. This lamp we know to be very active and rich in ultra violet rays. It cures rickets and produces the chemical reactions of ultra violet radiations. We have treated six rats by their method and in no case has their growth differed from the controls or in any way come up to the standard of Hume and Smith's rats. Taking another six rats we have kept four in a dim cupboard, heated to 28°C. by an iron pipe, and two in the cool, light laboratory. Two of those in the cupboard and one in the laboratory have had irradiated air treatment. The two in the laboratory have grown the best. The irradiated air has made no difference to the growth curves. We also tried on four pairs of rats the action of the following substances following as far as possible Hume and Smith's technique. (1) Ozone, (2) dilute  $\text{NO}_2$  gas, (3) air in glass jars that had been exposed to an X-ray tube and was presumably ionised, (4) cigarette smoke. Ozone is known to be produced by the light of a mercury vapour lamp acting on air;  $\text{NO}_2$  might be produced by the same agency acting on impure air, air containing traces of ammonia for instance. Cigarette smoke was taken as a more or less mild irritant and also because it might contain some ionised air produced by the act

of combustion. Without here going into any further detail, we were unable to stimulate the growth of rats by any of these methods. We have also failed to influence rickets in young rats fed on a deficient diet by exposing them daily for thirty minutes to the air led from the enclosure of mercury vapour lamp into their box, while exposure to the light of the lamp produced the well-known curative effect shown by Hess and others.

### **The metabolic behaviour of inositol in the developing egg.**

By J. NEEDHAM.

Of the metabolic relationships which may link the cycloses to other chemical substances in the animal body, practically nothing is known. Indications of a connection between inositol and carbohydrate metabolism have been sought for without success; and some workers have concluded that inositol plays no rôle of physiological importance, only appearing in the body because much of the phosphorus of the food is in the form of phytin.

In 1909 Klein(1) reported that inositol was absent from the unincubated egg of the hen, but was to be found in considerable quantity in the embryo of the 20th day. Now if inositol is synthesized in the egg, it is probably also synthesized in the body, and, furthermore, synchronous changes in the amounts of other substances in the developing egg may well be considered to be evidence of metabolic connection. In view of these possibilities, it was resolved to compare the behaviour of inositol with that of other bodies during incubation; and this communication is a preliminary report of the first part of the work.

150 eggs, laid by hens of the same breed, were taken and incubated for 21 days, samples being withdrawn every three days. Inositol was estimated by the method recently described by the author(2), and sugar by the Wood-Ost procedure. In the first place, Klein's failure to find inositol in the fresh egg was not confirmed; an egg on the first day after laying contains 6.2 mgs. p.c., mostly in the white. Thereafter, the total inositol-content increases to a maximum of about 35 mgs. p.c. by the 10th day, but drops again to under 20 mgs. p.c. by the 15th day. From then onwards the rise is steady and uninterrupted, reaching a level of over 60 mgs. p.c. at the time of hatching. The amounts in embryo and yolk-sac, calculated separately, follow these variations, but, after the 20th day the inositol increases much less rapidly in the embryo than it does in the remainder.

The sugar, on the other hand, estimated as total carbohydrate after hydrolysis with 10 p.c. HCl, showed no kink between the 8th and 17th days, but remained practically constant throughout until a sudden rise occurred on the last day of incubation.

From the increase of inositol, it must be concluded either that synthesis is taking place, or that it is being liberated from some inositol-containing substance. Since Plimmer and Scott<sup>(3)</sup> (1909) showed that the inorganic phosphorus of the egg increases to a very marked extent as development proceeds, it is not impossible that a phytin-like body may be gradually hydrolysed and thus yield free inositol. Further work is being done to test this point as well as to continue the general scheme of work outlined above on the metabolism of the developing egg.

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#### The relationship of the "chronaxie" of muscle to the size of the stimulating electrode. (*Preliminary Report.*) By HALLOWELL DAVIS.

Ever since Lapicque, Lucas, and others first published strength-duration curves for the electrical excitation of striated muscle there has been a contradiction as to the value of the *chronaxie* found for this tissue. The various workers have been in good agreement as to motor nerve, whose *chronaxie* is of the order of 0.0003 sec., varying somewhat with species of animal, function of nerve, size of fibre, temperature, etc. For striated muscle, however, Lapicque and his school find a *chronaxie* essentially the same as that of its motor nerve; while Lucas found it approximately ten to twenty times as long. The techniques of the two workers differ in that Lapicque stimulates the muscle *in situ* with a small silver wire electrode, while Lucas used the excised muscle in his "fluid electrodes." More recently Jinnaka and Azuma<sup>1</sup> have applied the pore electrode method for the stimulation of a single muscle fibre to the study of this problem, and have found values in general agreement with Lapicque.

A repetition of experiments by these various methods on the pelvic end of the sartorius of the frog shows that the different values are characteristic of the different techniques. Whether the muscle is excised or is *in situ* with the circulation active is of little importance, provided

<sup>1</sup> *Proc. Roy Soc. B.* 1921.



the excised tissue is bathed in a fluid of proper ionic concentrations. The important variable is the size of the electrode at which stimulation occurs. A small electrode, such as pores from  $3\mu$  up to  $75\mu$  in diameter or the contact of a metal wire, gives short *chronaxies* from 0.0002 sec. to 0.0005 sec., while fluid electrodes of the Lucas type give *chronaxies* up to 0.02 sec. By varying the size of the effective electrode nearly the whole range of intermediate values can be covered.

It has not yet been possible to make any satisfactory quantitative correlation between size of electrode, etc. and the duration or current strength values of the exciting current; but further experiments to this end are in progress. The long *chronaxie* of Lucas depends on low threshold values and long latent periods which appear when the current enters the tissue over a large area, but their exact significance is not yet clear. The pore electrodes resemble a little more closely, in point of size at any rate, the physiological method of stimulation, i.e. by the nerve, and incidentally give much smoother curves and more reproducible values than the fluid electrodes: but until the laws governing the wide variations can be worked out it does not seem possible to speak of any single *chronaxie* as characteristic of a given muscle.

### **Insulin and inositol.** By J. NEEDHAM, W. SMITH, and L. B. WINTER.

In recent times, since the true chemical nature of inositol has been discovered, a number of attempts have been made to demonstrate some connection between carbohydrate metabolism and the metabolism of this substance. From a study of the formulæ of the sugars on the one hand, and of the cycloses on the other, it has always seemed probable that they might be related. Nevertheless, all the work which has been done on this subject has had uniformly negative results; and absolutely no convincing evidence is at present available for a belief in their metabolic relationship.

The recent work of Dudley and Marrian<sup>(1)</sup> (1923) pointed to the need for reopening the question. Was it possible that some of the sugar which disappeared from the liver, blood, and muscles, during the convulsions following on the injection of insulin, might be transformed to cyclose? The experiments here recorded were accordingly carried out. Rats, similar as far as possible in age and development were taken, and insulin injected into some of them. When the convulsions came on, all were killed, and the total body-content of inositol estimated by the method already described by one of us (J. N. 1923)<sup>(2)</sup>. It was found that,

relatively, the insulin rats contained 50 p.c. more inositol than the normal ones. But, absolutely, owing to the very small amounts of inositol present in tissues, the increase was only an average of 4.2 mgs. per rat, so that it would not account for more than a fraction of the sugar, supposing a direct transformation to take place.

From the above facts, one of two conclusions is justifiable. Firstly, the increase of inositol might represent a side-reaction, as it were, in the removal of the sugar; or, secondly, it might be considered to be a secondary effect consequent upon the convulsions themselves. As nothing is yet known of the variations which inositol may undergo during muscular contraction, any definite preference is perhaps premature. Further work on these points is being carried out.

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### **Contraction of a drop of water by change of reaction.**

By R. BRINKMAN and A. VON SZENT GYÖRGYI.

It has become evident that interfacial phenomena must play an important rôle in the intimate mechanism of muscular contraction, as this developed itself from the more primitive modes of cellular mobility. It has also been made probable that the initial production of acid is responsible for the sudden change of surface-energy, but there is as yet no experimental explanation of the manner in which the liberated acid can produce a sudden increase of interfacial tensions. The object of this note is to show how a drop of water in a medium of petrol-ether contracts itself suddenly by increase of interfacial tension, if the reaction is changed from slightly alkaline to acid.

The influence of inorganic acids and bases on the pure water-air surface is very small, but this is not the case if we take a water-petrol-ether interface. Determination of the interfacial tension at this border by means of the torsion balance method shows that the tension is considerably lowered by a very small concentration of NaOH, and that it is raised immediately to its former dynamic value by acidulation with a small amount of HCl. It is a further important fact that this interfacial tension (which bears much more resemblance to the biological interfacial tensions than the commonly examined water-air tension does) is decreased very markedly by the gases oxygen and carbon dioxide.

By a very simple arrangement it is possible to demonstrate the significance of these facts for the surface-tension theories of contractility. The

arrangement is seen in Fig. 1. A small copper cylinder, which has been thoroughly cleansed and with a thin layer of water adsorbed to its surface, is immersed in a glass-beaker filled with petrol-ether. A large drop of water is placed on the concave top of the cylinder and a small copper disk, attached to an ordinary muscle-lever placed on the surface of the drop. When the lever is well balanced, the drop of water is stretched somewhat, and this stretching increases if a very small particle of solid NaOH is added to the drop, because of the lowering of interfacial tension by alkali. After some moments the tension has reached its lowest value,

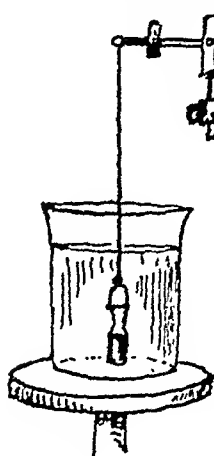


Fig. 1.

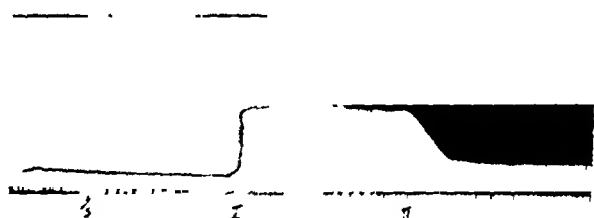


Fig. 2.

and now the lever is carefully brought into contact with a rotating smoked drum. Fig. 2 shows what takes place if the alkali drop is touched with a very small drop of HCl; the lever is suddenly raised by increase of tension of the drop (I). The drop remains in a contracted state till at (II) a minute droplet of a solution of  $\text{Na}_2\text{CO}_3$  is brought into contact with the acid drop of water. This results in the production of  $\text{CO}_2$  which is at once followed by a marked decrease of tension and elongation of the drop to its former length. In this way it is very easy to obtain an artificial myogram, by change of reaction at the water-petrol-ether interface, which may have interest for the theories on muscular contraction.

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